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PLATES 1 TO 3.

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INTRODUCTION.

The importance of acute lobar pneumonia and the need for means to prevent its ravages are now generally recognized. Among the infectious diseases pneumonia ranks next to tuberculosis as a cause of death, and in many of our large cities it kills more persons each year than does tuberculosis itself. In the public mind pneumonia is generally thought to affect mainly the very young and the old and decrepit. As a matter of fact, over half the cases occur between the ages of 20 and 50, during the period of greatest activity.

Although it occurs chiefly in endemic form, small and even large epidemics are not unknown. It was the most serious disease which threatened the success of construction of the Panama Canal, and its prevalence in certain regions where large numbers of susceptible workers are brought together, as in the mines of South Africa, renders it of great economic importance. Its importance as a cause of sickness and death among soldiers is not generally recognized, at least in this country, in spite of the fact that in our Civil War, aside from dysentery and typhoid, it caused far more deaths than any other disease. With improvements in knowledge of preventing and treating the two former diseases, pneumonia bids fair in the present war to lead all diseases as a cause of death. Judging from the experience in European armies, both in times of war and in times of peace, pneumonia is especially likely to attack raw recruits. The experience among the small number of troops on the Mexican Border, where pneumonia occurred in epidemic form, should be a warning of what is likely to happen in our national army when large numbers of susceptible men are brought together during the winter months.

Up to a few years ago the problem of preventing the spread of pneumonia or reducing its destructive effects seemed almost hopeless. The fact that bacteria apparently identical with those causing pneumonia are found in the mouths of many normal persons seemed to render any proposed measures for the prevention of infection well-

nigh useless. Attempts to cure the disease by the serum of immune animals had also given very inconclusive or negative results.

During recent years there has been in progress in the Hospital of The Rockefeller Institute a study of acute lobar pneumonia with the special object of improving methods of treatment. Realizing that such improvements are most likely to follow increase in knowledge of the pathogenesis of the disease and especially knowledge concerning the etiologic agent itself, much time has been given to the study of the pneumococcus, to the study of its distribution, which is so necessary for a knowledge of the epidemiology of the disease, and especially to a study of its immunity reactions, in order that methods for specific treatment might be obtained. As a result of this study, certain observations have been made and methods have been devised which have a direct practical bearing upon diagnosis and treatment. These observations have been published from time to time in papers appearing in *The Journal of Experimental Medicine* and elsewhere. At the present time of emergency and stress, however, it seems desirable that whatever is of practical value in this work should be made easily accessible, and, with the hope of meeting this need, this monograph has been prepared.

In the studies which have been carried on at the Hospital of The Rockefeller Institute, attention has been confined to pneumonia of the lobar type, due to *Diplococcus pneumoniæ*, though certain of the results obtained are undoubtedly applicable to lobular infections of the lung and to other pneumococcus infections as well. It is our purpose here, however, to consider only the application of bacteriologic and immunologic facts to the former disease.

It is not intended to present a complete description of this disease, the clinical features of which are well known. A brief general review of the bacteriology of pneumococcus, however, is included; but no attempt has been made to present a complete review of the entire subject. Much work which is undoubtedly important has not been discussed, and the writers have aimed chiefly to present the results of their own work and that of their associates in The Rockefeller Institute, and to describe the technique that has been there developed in the etiologic diagnosis and specific treatment of this disease. It is hoped that this monograph may be of aid to the

worker in the clinical laboratory who assists in diagnosis, to the public health worker who is attempting preventive measures, to the manufacturer who prepares immune serum, and to the clinician who carries out specific treatment. To meet all these requirements in a small space has demanded that the material be very much condensed and that, as far as possible, theoretic discussions be omitted. On the other hand, the effort has been made to describe methods accurately and with considerable attention to detail, in order that they may be employed even by those without very extensive bacteriologic training. It has been thought best to err on the side of too minute description of details and, in many cases, technical descriptions of very simple procedures, that are undoubtedly quite familiar to trained laboratory workers, are included.

The problems concerning pneumonia which remain to be solved are numerous, and the difficulties of successfully applying serum treatment to all forms of pneumonia still seem almost unsurmountable. Nevertheless, we now possess considerable knowledge concerning the mode of spread of the disease, more knowledge indeed than we have of some other diseases that are thought preventable, and we have perfected methods for successfully treating at least a part of the cases. We believe that there exists at present not only the justification for, but also the duty of attempting to apply these facts on a larger scale, especially at this time when special danger threatens a large part of our most valuable population, and when it is so important that human life be conserved. The application of these facts, however, cannot be effectively made in a crude haphazard manner. Sufficient supplies of serum of standard potency must be available and public health laboratories, hospital laboratories, and army field hospital laboratories must be equipped to carry out the necessary procedures. In addition, there is needed a very close cooperation between the laboratory workers and those actually engaged in the care of patients, and the latter, as well as the laboratory workers, should have knowledge of the principles concerned and of the laboratory methods employed.

DEFINITION AND ETIOLOGY.

Acute lobar pneumonia is an acute infectious disease, the characteristic pathologic feature of which is a uniformly diffuse, exudative inflammation of entire portions of one or more lobes of the lung. It has long been a question of dispute, however, whether the definition of the disease shall be based on pathologic, etiologic, or clinical features. In our opinion, as far as prevention and cure of the disease are concerned, it is of the greatest importance that the chief stress should be laid on the etiologic agent. Lobar inflammation of the lung may undoubtedly be caused by a number of different bacteria. The vast majority of the lesions, however, are caused by varieties of *Diplococcus pneumoniæ*. Among 529 cases diagnosed from the clinical and pathologic features as acute lobar pneumonia, the following were the etiologic agents concerned:

Diplococcus pneumoniæ	454
Friedländer's bacillus	3
Bacillus influenzæ	6
Streptococcus pyogenes	7
Streptococcus mucosus	1
Staphylococcus aureus	3
Cases of mixed infection with combinations of *Staphylococcus aureus*, Friedländer's bacillus, *B. influenzæ*, *Streptococcus pyogenes*, and *Streptococcus viridans*	6
Undetermined (most of them occurring before accurate methods for determining the etiologic agent had been devised)	49
Total	529

It is not important to discuss here whether the pulmonary lesions due to *Diplococcus pneumoniæ* are identical with those produced by other microorganisms. In typical cases, possibly in all, differences are present. At the present time, however, the differences in lesions cannot be detected with certainty during the life of the patient. The cases due to different varieties of bacteria are clinically indistinguishable, though the typical cases due to certain organisms

may present certain fairly characteristic features. With further study of large series of cases in which the etiologic agent in the individual case is carefully determined, it may be possible to devise methods for making a differential diagnosis on clinical grounds. At present we must rely on the isolation and identification of the organism concerned in each individual case. While we recognize, therefore, that lobar pneumonia may be due to a number of different organisms, in our present study we prefer to limit the discussion to that form due to *Diplococcus pneumoniæ*. As will be shown later, all pneumococci are not identical, but may be differentiated into several different types. This fact is of fundamental importance in all that concerns prevention and treatment of this disease, and, therefore, we believe that from the clinical standpoint, as well as from the bacteriologic, the cases due to the different types of pneumococci, as well as those due to other varieties of bacteria, should be considered distinct clinical entities. Instead of considering lobar pneumonia one disease, we should consider it a group of diseases. At present we can distinguish no constant clinical or pathologic differences among the cases due to the different types of pneumococci, though certain observations suggest that a clinical differentiation, even of these cases, may not be entirely impossible. Such a separation of the cases of pneumonia is entirely analogous to what we have become accustomed to in the typhoid-paratyphoid group of infections. Clinically all the cases due to this entire group of organisms are very similar. Their differentiation, however, has become necessary for epidemiologic and therapeutic reasons. It is true that the different types of organisms in the enteric group possess slight cultural differences in addition to immunologic ones, while the different types of pneumococci differ from each other, as far as we can detect at present, almost entirely in their immunity reactions. These latter differences, however, from the standpoint of prophylaxis and treatment, are much more important than cultural distinctions.

DIPLOCOCCUS PNEUMONIÆ.

In 1881 Pasteur (21) described an organism, recovered from the saliva of a case of rabies, which produced a fatal septicemia in rabbits and which he designated *microbe septicémique du salive.* Almost synchronously, but quite independently, Sternberg (27) in this country reported the occurrence of a similar coccus in the saliva of healthy individuals. The microorganism described by these two investigators is now recognized as pneumococcus, or *Diplococcus pneumoniæ.* At the time these observations were made, however, the pneumococcus was not known to be associated with disease in man. It was not until the systematic investigations of Fraenkel (4) several years later (1884–85) on the etiology of pneumonia, and the report of Weichselbaum (34) on the occurrence of the pneumococcus in the blood, organs, and exudates of a large series of cases of this disease, that the causal relationship of this organism to lobar pneumonia was established.

The original discovery of pneumococcus in the saliva of healthy individuals, and the later investigations which proved this same organism to be associated with one of the most fatal of the acute infectious diseases in man, forecast the wide distribution which this organism is now known to possess. The fact that pneumococcus may vegetate as a harmless saprophyte in the mouth secretions of normal persons, and that this same organism may give rise to severe and fatal infections in man is also foretold in the early history of its discovery. Subsequent work by numerous investigators has abundantly confirmed this curious paradox, but the interpretation of its significance in the epidemiology and specific therapy of pneumonia has been made possible only by the recognition of the biologic relationships of pneumococci by means of immunity reactions.

Morphology and Cultural and Other Biologic Characteristics.

Morphology.—Pneumococcus is a Gram-positive coccus which in the exudates of infected animals and on artificial media is typically a lance-shaped diplococcus. Variations in the size and form of the cocci may be noticed, however, in different cultures and even in the individual organisms present in the same culture. Oval, round, and

elongated bacillary forms may occur. In young, actively growing broth cultures of pneumococcus, chain formation is common, while in the same culture, after 18 hours' incubation, the organisms are usually arranged in pairs with only an occasional short chain. In older cultures of pneumococcus, the organisms often appear Gram-negative, swollen, with degenerated and involuted forms, resulting from disintegration and death of the cells. Pneumococcus is readily stained by all the usual aniline dyes. It possesses no flagella, is non-motile, and does not form spores. Capsules are easily demonstrable in animal exudates and, by appropriate methods, may be found in cultures from media enriched with blood or serum. The capsular substance is most marked in recently isolated virulent strains, and is readily lost on prolonged cultivation. In *Pneumococcus mucosus*, Type III, the capsule is so pronounced as to be frequently demonstrable by Gram's stain, by which method it appears as a negatively staining halo about the Gram-positive cell body. The exact significance of the capsule of pneumococcus is not known. That it may serve as a protective mechanism of the organism and that it may in some way be related to virulence, is suggested by the fact that capsular development is always much more marked when the organism is grown in animal tissues in which presumptively there is some opposition to its development. Study of a large number of strains of pneumococci shows that there are average differences in capsular development between Types I, II, III, and IV (page 17). Although in some strains of Type IV pneumococci capsular formation is well marked, in others it is quite irregular and at times impossible to demonstrate, even under the most favorable conditions. Type I pneumococci usually show well formed capsules, and in Type II pneumococci the capsules are quite easily demonstrable and are generally somewhat larger and denser than in Type I. In Type III pneumococci capsules are always well developed, are very large in comparison with the size of the organisms, and may appear as voluminous envelopes about the cell bodies. In our experience the amount of capsular development seems to parallel the virulence of these organisms for man, as measured by the mortality occasioned by the different types in human infection. In addition, it seems to be true that the greater the amount of capsular development, the less the amount of passive protection afforded by immune serum.

Cultural Characteristics.—While growth may be obtained on the usual simple media, pneumococcus is, nevertheless, sensitive to slight variation in composition and reaction of the media. On media prepared from meat extract, growth is irregular and at best sparse and delicate. The pneumococcus colony on the surface of meat infusion agar, which has been enriched by the addition of a small amount of sterile defibrinated rabbit blood, presents certain characteristic features. The colony is small, moist, translucent, discrete, and round, with well defined edges, greenish in color, with a checker-like or ringed topography. The colony is finely granular, with a darker center, surrounded by lighter ringed areas. Pneumococcus on blood media produces a greenish zone of discoloration about the colonies.

In broth pneumococcus grows diffusely, producing a uniform turbidity without pellicle and with a slight flocculent sediment. The turbidity of broth cultures begins to clear up after 24 hours' incubation, corresponding to the period of beginning autolysis and the passing into solution of the disintegrating bacterial protein.

Gelatin is not liquefied; this medium, however, is not well adapted for the cultivation of pneumococcus, since no growth of this organism occurs at temperatures below 25°C.

In milk and inulin serum water pneumococcus produces acid and coagulation of the medium. Inulin fermentation by pneumococcus is of differential value in distinguishing this organism from streptococcus. In studying the fermentation of inulin by pneumococcus, various irregularities are encountered. Although fermentation is the rule, it is not uncommon to find strains that fail to ferment this carbohydrate, and, in addition, the same strain may ferment it at one time, and fail to ferment it at another. The failure to ferment inulin in many instances may be due to the fact that not all preparations of inulin are fermentable by pneumococcus, and not infrequently different lots of media, prepared from the same inulin, vary in their suitability for growth. Therefore, while inulin fermentation is of value in differential diagnosis of pneumococcus from other organisms, this reaction should be considered only as confirmatory evidence.

The addition of simple carbohydrates to culture media enhances the initial growth of pneumococcus. However, the amount of acid

formed by pneumococcus in sugar-containing media quickly reaches a point at which growth is inhibited and the organisms rapidly die in the products of their own metabolism. Fermentative reactions of certain bacteria on selective carbohydrate have, in some instances, furnished a basis for differential classification. A study of a large number of strains of pneumococcus of known types on a series of different carbohydrates has so far failed to show any fermentative reaction which would afford a biochemic differentiation corresponding to the immunologic classification of the four types of pneumococcus.

Temperature and Oxygen Requirements.—Pneumococcus is not sensitive in its oxygen requirement, and growth occurs equally well under aerobic and anaerobic conditions. The optimum temperature for cultivation is 37°C., the limits of temperature range being 25–41°C. The thermal death-point of pneumococcus as determined by Sternberg is 52°C. for 10 minutes. The viability of pneumococcus on artificial media is relatively short. It has long been a matter of comment that even on suitable medium of proper reaction pneumococcus sometimes fails to grow. This is especially common when an attempt is made to grow the pneumococcus in meat infusion broth. In making transfers from broth to broth it is usually necessary to increase the amount of inoculum in proportion to the amount of broth in which the organism is to be grown (R36).[1] The usual amount of culture that it is necessary to transfer in order to insure growth is about 0.1 cc. to 5 cc. of broth. It is usually easy to start growth in broth if the inoculation is made from infected blood. A loop of heart's blood of a mouse dead of pneumococcus septicemia often suffices to initiate growth in large amounts of broth, and always succeeds in amounts of 5 to 25 cc. of the medium. On the other hand, the transfer from broth to broth of pneumococcus recently isolated is more difficult and usually requires a considerably larger inoculation. As a strain becomes accustomed to growth in broth, reinoculations into this medium grow with more regularity, although always requiring the relatively large inoculum already referred to. Chesney (2) has shown that transfers of pneumococcus made during the period of maximum rate of growth grow readily and without the latent period of de-

[1] *R* before reference numbers indicates that the title appears among the papers by the Staff of the Hospital of The Rockefeller Institute (page 106).

velopment which is characteristic of older cultures. Kirkbride (6) has further shown that when transfers in broth are made at 8 hour intervals, pneumococcus maintains its animal virulence for long periods of time, whereas cultures made at 24 hour periods rapidly lose their virulence.

Viability.—The addition of defibrinated rabbit blood to media not only enhances the growth of pneumococcus, but prolongs the life of the culture. Cultures of pneumococcus in blood broth preserved in the ice box remain viable for several weeks. One of the best methods for preserving pneumococcus cultures is that devised by Heim (5), by storing the dried spleens of infected mice. At the time of autopsy the spleen of the infected mouse is removed and transferred to a small sterile glass tube. The infected organ is quickly dried in vacuum and the desiccated tissue kept sealed in the cold, unexposed to light. Pneumococcus thus preserved maintains its viability and virulence for months. The culture may be recovered by grinding up the desiccated spleen in a sterile mortar with a little broth and injecting the organ emulsion intraperitoneally into a mouse, from which the pneumococcus can in turn be recovered in culture. In selecting spleens for preservation, care should be taken to choose those from animals in which no mouse typhoid infection exists. Mouse typhoid is a common infection in mice, frequently responsible for the death of large numbers when it becomes widespread. Mice which are apparently healthy frequently carry the bacillus of mouse typhoid in the intestine, and when these animals are experimentally infected with pneumococcus, the typhoid organism becomes active and invades the blood, tissues, and body cavities. It is difficult to obtain pure cultures of pneumococcus from such mice because of the rapidity and vigor with which *Bacillus typhi murium* grows on artificial media, and because of its great virulence for these animals.

Bile Reaction.—Neufeld (13) first observed the fact that pneumococcus in the presence of bile undergoes solution. Bile solubility is now recognized as a distinctive biologic character of pneumococcus, and serves to differentiate it from closely allied organisms, such as streptococcus. The active substance responsible for the lytic action of bile on pneumococcus is cholic acid. In carrying out the test, whole bile or 10 per cent solution of sodium taurocholate or sodium

glycocholate in physiologic salt solution may be used. One-fifth to one-tenth volume of bile will cause solution of an actively growing broth culture of pneumococcus. Heat-killed pneumococci lose their bile solubility. The presence of serum in pneumococcus cultures inhibits or completely arrests the lytic action of bile. Neufeld reports the insolubility of certain avirulent strains of pneumococcus. This phenomenon of insolubility, however, must be comparatively rare, since among several hundred strains of pneumococcus isolated by us from lobar pneumonia, none has failed to be dissolved by bile. In the diagnosis, then, of virulent pneumococcus, considerable reliance may be placed on the bile reaction.

Virulence.—The virulence of pneumococcus is variable; it is attenuated by prolonged cultivation on artificial media, and increased by animal passage. The virulence of pneumococcus for one animal species does not necessarily imply similar invasiveness for the tissues of another. Pneumococcus recovered from lesions in man does not invariably possess increased virulence for susceptible animals. Pneumococci isolated from the blood of pneumonia patients, as a rule, are of such virulence that 0.000001 cc. of broth culture kills white mice in less than 36 hours. On the other hand, strains have been recovered from the same source which possess only moderate virulence, failing to kill white mice in much larger doses. The study of the virulence of pneumococcus isolated from the circulating blood during lobar pneumonia indicates that in general the intoxication is more severe and the prognosis graver, the greater the virulence of the infecting pneumococcus. Since, however, virulence can only be experimentally tested in a different species, and since the defensive mechanism of the host is variable, no direct measure of virulence in man is available. However, as will be shown later in the discussion of the biologic types of pneumococci, infection with pneumococci of the various types results in differences in mortality which are fairly constant.

In addition to pneumonia, pneumococcus may give rise to inflammation of the upper respiratory tract and the accessory sinuses of the nose. Infection of the middle ear and invasion of the meninges may also be due to pneumococcus. Primary peritonitis of pneumococcus origin may occur, especially in young children. Focal infections, as

empyema, peritonitis, pericarditis, endocarditis, arthritis, and lung abscess due to pneumococci, may occur as sequelæ of pneumonia.

Toxin Production.—Pneumococcus is a highly pathogenic microorganism which is not known to secrete a soluble toxin, and whose harmful effects are supposed to be due either to the setting free of intracellular toxins, or to toxic split products formed by the disintegration of bacterial protein (R28), or possibly directly to the metabolic activities of the bacteria themselves (R29). When the bodies of pneumococci are dissolved either by the action of bile salts, autolysis, or by alternate freezing and thawing, the resulting bacterial solution is actively hemolytic for red blood corpuscles and is acutely toxic for animals (R18, R23). Intravenous injection of such solutions of pneumococcus produces death in rabbits and guinea pigs with acute symptoms resembling those of anaphylactic death. The hemolytic substance is thermolabile and its activity is lost by passage through a Berkefeld filter, and destroyed by proteolytic digestion with trypsin. Both the hemolytic and toxic properties of this substance are influenced by the same measures, and the potency of both is to some degree dependent upon the virulence of the pneumococcus from which the substance is derived. The lytic action of the hemotoxin, as well as its harmful effects in animals, may be inhibited by cholesterol and partially neutralized by immune serum. Neutralization by antipneumococcic serum, however, is incomplete and not strictly specific. Pneumococcus hemotoxin is not simply a product of autolysis, but undoubtedly exists preformed in the bacterial cell as a hemolytic endotoxin.

The production of methemoglobin by pneumococcus is another phenomenon the occurrence of which may in some way be concerned with the harmful effect of this organism in disease. The transformation of hemoglobin into methemoglobin by pneumococcus occurs only in the presence of free oxygen and the living bacterial cell. Culture filtrates and bacterial extracts of pneumococcus, when free from living organisms, do not induce the methemoglobin reaction.

Precipitable Substance (R6, R7).—Pneumococcus during the early stages of its growth forms a readily soluble substance which diffuses into the culture medium *in vitro*, and in human and animal infections is present in the circulating blood, whence it passes through the kid-

neys into the urine. This specific substance appears in the culture medium in considerable amounts as soon as the pneumococci start to grow. Its early presence is not attributable to the death and subsequent disintegration of the bacterial cell, but it is due to the extrusion into the medium of bacterial substance during the life processes of the organism.

One of the chief points of interest in the discovery of the soluble substance of pneumococcus is that it raises the question whether this substance is in any way responsible for the intoxication which attends pneumococcus infection. Studies to ascertain the answer to this question are being carried on at the present time, but have not yet progressed to the point at which a definite answer can be given. It may be said, however, that its toxicity is in no way comparable with that of diphtheria toxin. On the other hand, it possesses a degree of toxicity which, exhibited throughout the course of an infection, might account for the signs of intoxication in lobar pneumonia.

Differential Diagnosis.

Although a differential diagnosis is not always simple, pneumococcus possesses certain cardinal characteristics by virtue of which, in most instances, it may be readily distinguished from closely allied organisms, as streptococcus. Pneumococcus is bile-soluble, possesses a capsule, ferments inulin, is extremely pathogenic for mice, and on blood agar forms a small, moist, flat, ringed, checker-like colony with a greenish zone of methemoglobin about it. Moreover, as will be discussed later, the various types of pneumococci react specifically with their homologous immune sera. Streptococcus, on the other hand, is not bile-soluble, does not ferment inulin, is less virulent for mice; on blood media the colony is more opaque and raised, drier, and more coarsely granular, without the surface markings or ringed topography characteristic of pneumococcus, and is surrounded by a zone of either hemolysis or green pigmentation.

Considerable confusion still exists in bacteriologic literature regarding the identity of so called *Pneumococcus mucosus* and *Streptococcus mucosus*. *Pneumococcus mucosus*, or Type III pneumococcus, possesses the characteristics already described as typical of pneumococcus; it is bile-soluble, ferments inulin, possesses a voluminous cap-

sule, is extremely virulent for mice, and produces greenish discoloration of blood media. *Streptococcus mucosus*, on the other hand, while having a similar mucoid, heaped up, confluent colony, and possessing a capsule, is, however, not bile-soluble, does not split inulin, is not so pathogenic for mice, and has a greater tendency to produce hemolysis on blood agar. Finally, this organism, like other streptococci, does not react specifically with Antipneumococcic Serum Type III.

Biologic Classification.

With recent advances in bacteriology and the knowledge gained from attempts to apply the principles of bacterial immunity to human disease has come the recognition of the fact that, in addition to the grosser classification of bacteria, certain finer characteristics exist which serve to differentiate still further members of the same bacterial species. Through the work of Neufeld (16) and his associates, it was demonstrated that all races of pneumococci are not identical as far as their immunologic reactions are concerned. When the study of pneumonia at the Hospital of The Rockefeller Institute was undertaken, a large number of races of pneumococci from cases of pneumonia was isolated and the immune reactions of these were carefully studied (R33, R20, R35). Animals were immunized to each of these strains, and the immunity reactions of all of them to the various immune sera so obtained were studied. Not only was the agglutinability of the various races in each of the sera determined, but also the power of each of the sera to protect mice from infection with each of the strains. The results of these studies revealed the interesting fact that pneumococci fall into two general classes. The larger of these consists of pneumococci of Types I, II, and III, which comprise about 80 per cent of all strains encountered in disease, and which represent three apparently fixed types of highly parasitic organisms, each possessing common immunologic characters. Individual strains of Type I and Type II are characterized by the possession of immunity reactions common to all other members of the homologous group. A number of variants of Type II pneumococcus have been found (R4), a condition of considerable theoretic interest, the practical importance of which, however, is not sufficiently great to warrant detailed discussion here. Type III consists of *Pneumo-*

coccus mucosus, an organism distinguished from other types by morphologic and cultural, as well as immunologic differences, and related to other strains of the same type by common immunologic reactions (R38).

The smaller of these two main classes, which has been called Type IV, represents about 20 per cent of the strains isolated from cases of lobar pneumonia, and is the type most frequently encountered in the mouth secretions of normal individuals. Type IV pneumococci possess greater heterogeneity and consist for the most part of individual strains which are not interrelated. As originally described, this group was considered peculiar in that it seemed to comprise a heterogeneous series of independent varieties, none of which exhibited immunity reactions common to the other types or to each other. However, more recent study by Olmstead (19) of a large series of strains of Pneumococcus Type IV has shown that this group may contain smaller groups, the members of each one of which possess certain characteristic immunologic properties. These studies of Type IV pneumococcus are of interest and the results hold promise of practical application. At present, however, the work has not progressed to a point where practical use of it can be made, and hence it would be premature to discuss this group from the standpoint of specific therapy.

TABLE I.

Occurrence of Various Types of Pneumococcus in Lobar Pneumonia (454 Cases).

Pneumococcus type.	Incidence.	
		per cent
I	151	33.3
II	133	29.3
II (atypical)*	19	4.2
III	59	13.0
IV	92	20.3

*The incidence of atypical Type II pneumococci has been determined only during the last 2 years.

From Table I it is evident that Pneumococcus Type I is the most common cause of infection in man, giving rise to about one-third of the cases. Type II is next in frequency, and these two types together

give rise to over 60 per cent of cases of lobar pneumonia, at least in those localities in the United States in which studies of types of pneumococcus have been made. *Pneumococcus mucosus* (Type III) gives the lowest incidence in disease of the different types of pneumococcus. Type IV is responsible for about 20 per cent of cases, although the incidence of cases due to this organism seems to vary somewhat in different localities.

The accuracy with which these types may be differentiated and the constancy of their relative frequency in disease and health emphasize the importance of their recognition in clinical and epidemiologic studies. The exactness with which the large number of strains studied has conformed to type indicates the extraordinary uniformity and comparative fixity of the specific groups. These distinctive differences in immunologic properties of pneumococcus not only offer a reliable method for the more exact determination of the varieties of pneumococcus, but afford the only rational basis for the study of the epidemiology and immunotherapy of pneumococcal infections.

Culture Media and Staining Methods.

In the preparation of media suitable for growth of pneumococcus, attention should be paid to the following points:

1. All media should be prepared directly from infusion of fresh meat and not from beef extract.

2. Reaction of the media should be from 0.3 to 0.5 acid to phenolphthalein.

3. In the sterilization of the media, care should be taken to avoid excessive heating. Media should be sterilized by the Arnold method, 20 minutes on 3 successive days, and should not be autoclaved.

The following procedure has been found to give satisfactory results in the preparation of broth and agar for cultivation of pneumococcus:

Nutrient Broth and Agar.—1 pound of lean chopped beef is allowed to infuse in a liter of tap water over night on ice. The unfiltered meat infusion is boiled for 30 minutes, filtered through paper, and the loss by evaporation made up by the addition of water. 1 per cent peptone and 0.5 per cent sodium chloride are now added. 1.5 per cent agar may be added at this point if agar is desired. The mixture is allowed to boil for 2 minutes in the case of broth, or, if for agar, until the agar is dissolved. Titration to the neutral point with normal sodium hy-

droxide is then carefully carried out. Media are then boiled for 6 or 7 minutes, made up to volume, and filtered clear, and sterilized in the Arnold sterilizer for 20 minutes on 3 successive days. The media should finally titer 0.3 to 0.5 acid to phenolphthalein.

Blood Agar.—For enrichment purposes, the addition of a small amount of sterile defibrinated rabbit blood to media prepared as above is recommended. The addition of three or four drops of sterile blood to 4 or 5 cc. of agar or bouillon serves as ample enrichment and provides most suitable medium for the growth and preservation of pneumococcus cultures. Blood may be obtained either from the ear vein of a rabbit or by direct heart puncture. Drawn directly into a small sterile container with glass beads, the defibrinated blood can be preserved for a week or more, and the method is, therefore, economical.

Serum Water Medium for Fermentation Tests.—For the determination of inulin fermentation by pneumococcus, the following medium devised by Hiss is used, a positive reaction being indicated by the production of acid and the coagulation of the serum protein. Clear beef serum is added to 2 or 3 volumes of distilled water. Heat the mixture for 15 minutes in an Arnold sterilizer at 100°C. to destroy ferments present in the serum. Add 5 per cent aqueous litmus solution to a concentration of 1 per cent or an amount sufficient to give a deep blue color. Add inulin to the serum water to a concentration of 1 per cent. The inulin may be best sterilized by autoclaving at 15 pounds' pressure for 15 minutes. Sterilize the inulin serum water by the fractional method, 100°C.

Preparation of Bile for Testing the Solubility of Pneumococcus.—Fresh ox bile, obtained directly from the slaughter house, is autoclaved for 20 minutes, at 15 pounds' pressure. The bile is filtered to remove the precipitate formed on heating and again autoclaved. The sterile bile is then ready for use. Heat does not destroy the lytic action of bile. While different samples of bile vary in their content of the salts of cholic acid and hence in their ability to dissolve pneumococcus, for routine purposes the bile reaction may be carried out by the addition of one-fifth to one-tenth volume of whole bile to plain broth culture of pneumococcus.

Stains for Pneumococcus. Gram's Stain (Sterling's Modification).—

Gentian violet	5 gm.
Alcohol, 95 per cent	10 cc.
Aniline oil	2 "
Distilled water	88 "

The aniline oil and alcohol are mixed by shaking, and the distilled water is added. The gentian violet is ground in a mortar and the aniline solution added while grinding. The solution is stable 3 to 4 months and stains rapidly.

Gram's Iodine Solution.—

Iodine	1 gm.
Potassium iodide	2 "

Dissolve in 20 cc. of distilled water. Add 300 cc. of distilled water.

Safranin Counterstain.—10 per cent aqueous solution of a saturated alcoholic solution of safranin.

Hiss' Capsule Stain.—Preparations are best made by direct films from pneumococcus exudates. Dry in air and fix by heat. Stain for a few seconds with saturated alcoholic solution of fuchsin or gentian violet, 5 cc., in distilled water, 95 cc. Flood the slide with the dye and hold the preparation for a second over a free flame until it steams. Wash off the dye with 20 per cent aqueous copper sulfate solution. Blot; do not wash in water.

By this method the capsule appears as a faint blue halo around a dark purple cell body. Better results may frequently be obtained by omitting heat fixation and by washing off the dye with the copper sulfate solution as soon as it begins to steam. Water should not be applied at any stage of the procedure.

ETIOLOGIC DIAGNOSIS.

If we possessed no methods for specific treatment or for instituting specific preventive measures, the determination of the etiologic agent in each individual case of lobar pneumonia would not be of great importance. However, since it is now known that certain cases of pneumonia may be successfully treated with the serum of immune animals, provided that the organism used in preparing the serum is identical with that causing the infection, etiologic diagnosis becomes imperative. Practical and quite rapid methods have now been devised for the detection of the organism causing the disease, even for the differentiation of the specific types of pneumococci. This etiologic diagnosis is, however, not only of importance in relation to serum therapy, but has an important bearing on prognosis and is essential for acquiring knowledge concerning the epidemiology of this disease.

For the determination of the etiologic agent in cases due to other organisms than pneumococcus, the same general methods are applicable as in cases due to pneumococcus. In certain cases, as those due to Friedländer's bacillus, streptococcus, or staphylococcus, the microscopic examination of the sputum gives much information, and cultures of the sputum made on agar plates may show a great predominance of the organisms causing the disease. When the sputum from these cases is inoculated into a mouse, as in the method about to be described, these organisms outgrow the ordinary mouth saprophytes just as do pneumococci. Blood cultures from the patient, when positive, give, of course, clear evidence as to the nature of the infection.

In the cases due to pneumococcus it is important not only to determine that the infection is due to this bacterium, but it is also of prime importance to determine the specific type of pneumococcus causing the infection, since treatment with serum is only applicable in the cases due to Type I organisms.

The following is a description of the various procedures employed in the isolation of pneumococci and determination of the specific types:

Isolation of Pneumococci.

Organization of Laboratory.—In order that these methods may be of practical value, hospital and Board of Health laboratories should be so organized, if possible, that diagnoses can be made at any time, day or night. Arrangements for the rapid transmission of the specimen of sputum to the laboratory are of great importance, since the diagnosis must be made rapidly if the best results from serum therapy are to be obtained. After a moderate amount of experience by the laboratory worker, the method can be carried out with much rapidity, especially where a number of diagnoses are to be made at the same time; and where the work is properly organized, public health laboratories should not find that the introduction of this procedure into their routine adds a great burden. For the actual carrying out of the procedure, the comparatively simple equipment of any bacteriologic laboratory is sufficient. Facilities for the proper collection and microscopic examination of sputum, together with means for its inoculation into mice, and the simple requirements for mouse autopsy and bacterial cultivation are all the equipment necessary.

Collection of Sputum.—Care should be exercised in the collection of sputum to obtain a specimen from the deeper air passages, as free as possible from saliva. This can be done in practically all cases, even the most difficult, with a little persistence. The sputum is collected in a sterile Esmarch dish, or other suitable container, and should be sent at once to the laboratory for examination. When delay is unavoidable, the specimen should be kept on ice during the interval.

Microscopic Examination of Sputum.—Direct films of sputum are stained by Gram, by Ziehl-Neelsen, and by Hiss capsule stains. This serves to give an idea of the nature of the organisms present and an indication of the source of the sputum. Suitable lung specimens of sputum are relatively free, in most instances, from contaminating mouth organisms. It is frequently possible to identify Type III (*Pneumococcus mucosus*) organisms when they are present, as they possess very large, distinct capsules, staining by both Gram's and Hiss' methods.

Mouse Inoculation.—A small portion of the sputum, about the size of a bean, is selected and washed through three or four changes of

sterile salt solution in sterile Esmarch or Petri dishes to remove surface contaminations. When the sputum is too friable or when the specimen is relatively free from secondary organisms, this washing process may be omitted. In either event, the kernel of sputum selected is transferred to a sterile mortar, ground up, and emulsified with about 1 cc. of sterile bouillon or salt solution, added drop by drop, until a homogeneous emulsion is obtained that will readily pass through the needle of a small syringe. With a sterile syringe 0.5 to 1 cc. of this emulsion is inoculated intraperitoneally into a white mouse (Figs. 1, 2, and 3). The pneumococcus grows rapidly in the mouse peritoneum, while the majority of other organisms rapidly die off, with the exception of Friedländer's bacillus, *Bacillus influenzæ*, and occasionally *Micrococcus catarrhalis*, staphylococcus, and streptococcus. Pneumococcal invasion of the blood stream also occurs early. *Bacillus influenzæ*, if present, likewise invades the blood stream; other organisms, as a rule, do not. The time elapsing before there is sufficient growth of pneumococcus in the mouse peritoneum for the satisfactory determination of type varies with the individual case, depending upon the abundance of pneumococci in the specimen of sputum and the virulence and invasiveness of the strain present. It may be from 5 to 24 hours, averaging 6 to 8 hours with the parasitic fixed types I, II, and III. As soon as the injected mouse appears sick, a drop of peritoneal exudate is removed by means of peritoneal puncture with a sterile capillary pipette, spread on a slide, stained by Gram's method, and examined microscopically to determine whether there is an abundant growth of pneumococcus present. If there is an abundant growth of pneumococcus alone, the mouse is killed and the determination of type proceeded with. If the growth is only moderate, or if other organisms are present in any quantity, further time must be allowed until subsequent examination of the peritoneal exudate shows an abundant growth of pneumococcus. It should be emphasized that undue haste in killing the mouse is time lost in the end.

Mouse Autopsy (*Fig. 4*).—As soon as the mouse is killed or dies, the peritoneal cavity is opened with sterile precautions and cultures are made from the exudate in plain broth and on one-half of a blood agar plate. Films are made and stained for microscopic examination by Gram's stain and Hiss' capsule stain. The peritoneal exudate is then washed out by means of a sterile glass pipette with 4 to 5 cc. of sterile salt solution, the washings being placed in a centrifuge tube.

Cultures are then made from the heart's blood in plain broth and on the other half of the blood agar plate.

Determination of Types of Pneumococcus.

Agglutination Method.—When the pneumococcus is present in pure culture in the peritoneal exudate, the determination of type may satisfactorily be made by macroscopic agglutination tests, as follows: The peritoneal washings are centrifuged at low speed for a few minutes until the cells and fibrin contained in the exudate are thrown down. The supernatant bacterial suspension is transferred into a second centrifuge tube and centrifuged at high speed until the organisms are thrown out. The supernatant fluid is discarded and the bacterial sediment taken up in sufficient salt solution to make a moderately heavy suspension. The concentration of bacteria should be similar to that of a good 18 hour broth culture of pneumococcus. This suspension is used directly for macroscopic agglutination tests, being mixed with dilutions of immune serum in small test-tubes in equal quantities of 0.5 cc. each.

To obviate the difficulty that occasionally arises from the occurrence of Type IV strains that show cross agglutination in all three immune sera, the optimum dilutions of serum and the optimum incubation time that will surely identify all type strains and fail to give any cross agglutination reactions, have been determined on a large series of strains.[2] The results are shown in Table II.

TABLE II.

Determination of Pneumococcus Types by Agglutination.

Pneumococcus suspension 0.5 cc.	Serum I (1:20) 0.5 cc.	Serum II (undiluted) 0.5 cc.	Serum II (1:20) 0.5 cc.	Serum III (1:5) 0.5 cc.
Type I	++	−	−	−
" II	−	++	++	−
Subgroups IIa, b, x	−	+	−	−
Type III	−	−	−	++
" IV	−	−	−	−

Incubation for 1 hour at 37°C.

[2] The dilutions of immune serum indicated in both the agglutinin and precipitin tests refer only to the antipneumococcic serum in use at the Hospital of The Rockefeller Institute. As various lots of serum may differ in agglutinin and precipitin content, the concentration of serum required to assure specificity of reaction may vary somewhat from the figures given in Tables II and III.

From Table II it will be seen that a 1:20 dilution of Type I serum, making, with the addition of an equal amount of pneumococcus suspension, a final dilution of 1:40, a 1:20 dilution of Type II serum, making a final dilution of 1:40, and a 1:5 dilution of Type III serum, making a final dilution of 1:10, serve to agglutinate Types I, II, and III pneumococci respectively and fail to show any cross agglutination reaction with strains belonging to Type IV. It will further be seen that with 0.5 cc. of undiluted Type II serum, as well as with the 1:20 dilution, pneumococci belonging to the various subgroups of Type II may be identified and rapidly differentiated from Type II pneumococcus, in that they show partial to complete agglutination in undiluted Type II serum only, but not in the 1:20 dilution at the end of 1 hour's incubation at 37°C. For the determination of types of pneumococcus in the peritoneal washings, these serum dilutions give the most satisfactory and clear-cut results. Five small test-tubes are set up as follows:

Tube 1.	0.5 cc.	Serum	I (1:20)	+ 0.5 cc.	bacterial	suspension.
" 2.	0.5 "	"	II (undiluted)	+ 0.5 "	"	"
" 3.	0.5 "	"	II (1:20)	+ 0.5 "	"	"
" 4.	0.5 "	"	III (1:5)	+ 0.5 "	"	"
" 5.	0.1 "	sterile ox bile		+ 0.4 "	"	"

Tube 5, containing bile plus bacterial suspension, is for the determination of the bile solubility of the strain and for the differentiation of pneumococcus from streptococcus. The tubes are incubated in the water bath for 1 hour at 37°C. Agglutination of Pneumococcus Types I, II, and III occurs promptly in these serum dilutions and is specific. Rapid clumping of the organisms is seen to take place and may be brought out clearly by gentle agitation of the tubes. For the identification of atypical Type II pneumococcus, incubation is necessary, these strains showing partial to atypical agglutination in undiluted Type II serum at the end of 1 hour's incubation. If no agglutination occurs in any of the serum tubes and the organism is bile-soluble, it is classified as Type IV pneumococcus.

Precipitin Method.—The determination of pneumococcus types by macroscopic agglutination tests with the peritoneal washings is interfered with when other organisms are present in considerable numbers, as occasionally happens, and consequently a delay of 18 hours

or more may occur before the type of pneumococcus present can be established. To obviate this, Blake (R9) has devised the following method: It has been shown that pneumococcus during the period of active growth in broth cultures produces a soluble substance which gives a specific precipitin reaction with homologous antipneumococcic serum. The method described by Blake is dependent upon this phenomenon. The peritoneal exudate is washed out with 4 to 5 cc. of sterile salt solution in the manner already described under the agglutination method, and the peritoneal washings containing cells, fibrin, and bacteria are immediately centrifuged at high speed until the supernatant fluid is water-clear. The supernatant fluid is then pipetted off with care not to disturb the sediment, and is mixed in quantities of 0.5 cc. each with equal amounts of antipneumococcic serum in a series of small test-tubes as follows:

Tube	1.	0.5 cc.	Serum	I (1:10)	+	0.5 cc.	supernatant	peritoneal	washings.
"	2.	0.5 "	"	II (undiluted)	+	0.5 "	"	"	"
"	3.	0.5 "	"	II (1:10)	+	0.5 "	"	"	"
"	4.	0.5 "	"	III (1:5)	+	0.5 "	"	"	"

An immediate specific precipitin reaction occurs in the tube containing homologous immune serum, the other tubes remaining clear (Table III). Incubation is usually not necessary. Two tubes of Type II serum are used for the purpose of distinguishing between Type II pneumococcus and members of its subgroups, the former giving a precipitin reaction in both tubes, the latter only in the undiluted Type II serum. A negative reaction in all serum tubes indicates pneumococcus belonging to Type IV. The precipitin method has been tested with a large number of strains and has been consistently positive and specific with Pneumococcus Types I, II, and III, and consistently negative with Type IV. The presence of other organisms, together with pneumococcus, in the peritoneal exudate does not interfere with the reaction, and other microorganisms than pneumococcus produce no substance that might give a false positive reaction.

The results with Subgroup II pneumococcus have not been so satisfactory. Reference to Table III will show that pneumococci belonging in these subgroups give a precipitin reaction with undiluted Type II serum, but not with the 1:10 dilution, thereby being distinguished

TABLE III.

Determination of Pneumococcus Types by the Precipitin Method.

Supernatant peritoneal washings 0.5 cc.	Serum I (1:10) 0.5 cc.	Serum II (undiluted) 0.5 cc.	Serum II (1:10) 0.5 cc.	Serum III (1:5) 0.5 cc.
Type I	++	−	−	−
" II	−	++	++	−
Subgroups IIa, b, x	−	+	−	−
Type III	−	−	−	++
" IV	−	−	−	−

from typical Pneumococcus Type II. A number of Subgroup II organisms, however, have been encountered in which the peritoneal washings have failed to give a precipitin reaction with undiluted Type II serum. In the identification of the fixed parasitic types of pneumococcus, this occasional difficulty is of little practical importance from the point of view of treatment, as there is at present no specific therapy for cases of pneumonia due to organisms of these types. For purposes of classification and statistics, these organisms can be readily identified subsequently when pure cultures have been obtained.

Confirmation of Types.—The determination of the type of pneumococcus by examination of the peritoneal washings should be confirmed by macroscopic agglutination tests with a pure bouillon culture of pneumococcus obtained from the heart's blood at the time of mouse autopsy. The technique is the same as that employed in the agglutination tests on the bacterial suspension obtained from the peritoneal washings. A test for bile solubility should also be made.

Determination of Type III Pneumococcus by Morphologic and Cultural Characteristics.

Of the four types of pneumococcus, the third type, *Pneumococcus mucosus*, is the only one which can be identified in most instances by morphologic and cultural differences. *Pneumococcus mucosus* is usually somewhat larger, rounder, and less lanceolate than other types of pneumococcus. It possesses a large distinct capsule which stains readily with Hiss' capsule stain and which usually retains the pink counterstain with Gram's method. The peritoneal exudate

of a mouse infected with this organism is usually quite mucoid and sticky, and the colonies on blood media are moist, mucoid, and confluent. Occasional strains, however, are encountered in which these characteristics are not so well marked and which are not so easily distinguishable by cultural or morphologic differences from other types. Furthermore, Pneumococcus Type II occasionally exhibits fairly well defined mucoid characteristics. For these reasons the identification of Type III pneumococcus by morphologic and cultural characteristics is not always absolute, and the diagnosis should be established by immunologic methods, when Type III serum is available.

Determination of Types of Pneumococcus in Blood Cultures, Spinal Fluids, Empyema Fluids, and by Lung Puncture.

Blood Culture.—From a positive bouillon blood culture, 10 cc. are removed by pipette and centrifuged at low speed to remove the blood cells. The supernatant fluid is pipetted off and the bacteria are thrown down by centrifuging at high speed. The supernatant fluid is discarded and the bacterial sediment resuspended in sterile salt solution. The type of pneumococcus is then determined by macroscopic agglutination tests following the technique described above.

Spinal Fluids and Empyema Fluids.—Cultures are made by the methods ordinarily employed in culturing these fluids, and the type of pneumococcus is determined by the agglutination method, with the same technique as that applied to blood cultures. If desired, in addition to culturing the fluids, a portion may be centrifuged at high speed to throw down the pneumococci present, and the bacterial residue may be taken up in 1 cc. of sterile salt solution and inoculated intraperitoneally into a mouse.

Lung Puncture.—This procedure should be resorted to only when it is impossible to obtain a suitable specimen of sputum or a positive blood culture. In our experience this procedure is necessary only on rare occasions. Cultures of the material obtained by puncture are made in bouillon, and the determination of type is made by the same technique as that employed in the case of blood cultures.

Determination of Types of Pneumococcus by Means of Specific Precipitin Reaction in the Urine.

The fact has been mentioned that a certain percentage of patients suffering from lobar pneumonia excrete in their urine at some stage of the disease a soluble substance of pneumococcus origin. This substance gives a specific precipitin reaction with antipneumococcic serum corresponding in type to the organism with which the individual is infected. A study of 111 cases of lobar pneumonia and closely related respiratory diseases has shown that in 65 per cent of the cases of pneumonia due to Pneumococcus Types I, II, and III, this substance is present in the urine and can be detected by means of the appropriate antipneumococcic serum. The reaction is specific for the type of pneumococcus with which the individual is infected, and in no instance of respiratory disease due to organisms other than pneumococcus has a positive reaction been obtained. The soluble substance may appear in the urine at as early a period as 12 hours after the initial chill, or it may appear for the first time at a later stage of the disease. It may continue to be excreted for many days after recovery has occurred. It is the rule to find this substance in the urine when pneumococcus septicemia exists. Examination of the urine for the presence of this substance has proven of some value for the rapid determination of the type of pneumococcus with which the individual is infected. Methods for the performance of the precipitin test are therefore given in detail. This test, however, should not supplant the usual bacteriologic methods for the differential diagnosis of pneumococcus types, but is useful in that in a certain percentage of instances an immediate diagnosis can be made from an examination of the urine.

Method Using Unconcentrated Urine.—A specimen of urine is obtained as soon as possible and cleared by centrifuging. The clear urine is mixed in quantities of 0.5 cc. each with equal amounts of antipneumococcic serum of the different types in a series of agglutination tubes (Table IV).

The strength of the reaction may vary from an almost imperceptible cloud to a heavy flocculent precipitate. The reaction may occur in some instances immediately on the mixing of urine and serum, or

TABLE IV.

Determination of Pneumococcus Types by Precipitin Reaction in Urine.

0.5 cc. urine from:	Serum I 0.5 cc.	Serum II 0.5 cc.	Serum III 0.5 cc.
Type I pneumonia.....	+	−	−
" II "	−	+	−
" III "	−	−	+
" IV "	−	−	−

may require incubation in the water bath at 37°C. for 1 hour. Prolonged incubation, however, must be avoided, since bacterial growth under such conditions may obscure the test. In the precipitin reaction it is essential that all the reagents used, including the immune sera, should be water-clear. In case the reaction is negative or so faint as to be indecisive with the whole urine, the following method of concentrating the urine may be employed:

Concentration of Urine for Precipitin Test.—25 cc. or more of a 24 hour specimen of urine, with the addition of a few drops of acetic acid, are boiled down to a volume of about 5 cc., filtered through paper to remove any precipitate of albumin that may occur, and the filtrate is added to 8 to 10 volumes of 95 per cent alcohol. The precipitate which forms is collected by centrifuging, rapidly dried to remove the excess of alcohol, and the residue extracted with 2 to 3 cc. of salt solution, which redissolves the specific substance. Any undissolved material is removed by centrifuging and the clear salt solution extract is used for the precipitin test, as shown in Table IV.

LABORATORY AIDS IN PROGNOSIS.

Accurate prognosis in such an acute disease and one of such short duration as lobar pneumonia is very difficult. That the clinical picture may pass in the brief period of 24 hours from one that is apparently favorable to one of extreme urgency is a common observation. In the early stages of the disease it is almost impossible from the clinical picture alone to predict the outcome, and even in cases ending fatally, it is frequently difficult, even during the last days, to say what the result will be.

In the course of the studies on lobar pneumonia at the Hospital of The Rockefeller Institute some facts have been determined which are of value in estimating the probable severity of any case of pneumonia, and in predicting the result. Studies of the bacteriology of a large series of cases of lobar pneumonia have shown that the type of pneumococcus with which the individual is infected plays a part of considerable importance in the probable outcome of the disease. In spite of certain reports to the contrary, our experience has led us to believe that the presence of pneumococcus in the blood during lobar pneumonia indicates a severe infection and is of bad prognostic import. It has already been stated that during the course of lobar pneumonia there appears in the urine a soluble substance of pneumococcus origin. The quantity of this substance can be followed from day to day, and the amount excreted seems to be directly proportional to the severity of the infection. In occasional cases the blood picture is of value in forecasting the outcome of the disease.

Relation of Type of Pneumococcus to Mortality.

A study of a large number of strains of pneumococcus and the outcome of the disease in individuals infected with these strains has shown that the different types of pneumococcus vary in their virulence for man. In Table V are shown the percentage incidence of the different types of pneumococci in 100 cases of lobar pneumonia and the average mortality in those untreated with serum.

TABLE V.

Incidence of Various Types of Pneumococcus and Resulting Mortality.

Type of pneumococcus.	Incidence.	Mortality.
	per cent	*per cent*
I	33	25
II	31	32
III	12	45
IV	24	16

The statistics given in Table V approximate the facts as closely as they can be ascertained at the present time. Organisms of Type IV have in our experience been found to possess the lowest virulence of the different types for human beings. Type III pneumococcus, although it is the cause of the smallest percentage of cases, nevertheless gives rise to a high mortality, and the outlook for a patient infected with Type III pneumococcus is comparatively bad. Pneumococci of Types I and II are responsible for about 64 per cent of all cases of lobar pneumonia and are of relatively high virulence for man, the largest number of deaths in lobar pneumonia being due to these organisms. The mortality in Type I infection is 25 per cent, and in infections with Type II pneumococcus about 32 per cent. These two types together are responsible for approximately 62 per cent of all deaths from lobar pneumonia. It is evident, therefore, that the outcome of any case of lobar pneumonia is influenced by the type of organism with which the individual is infected, and knowledge of the type of pneumococcus causing the infection is therefore of value in prognosis. It must be borne in mind, however, that there is considerable variation in the severity of the individual cases due to the different types of pneumococcus.

Specific Precipitin Reaction in the Urine.

In the course of our studies it has been found that pneumococci of Types I, II, and III during the active period of growth form a soluble substance. Type III pneumococcus forms the largest amount of this substance, Type II somewhat less, and Type I the least. The elaboration of this substance by Type IV pneumococcus has not been

studied because of lack of specific sera for this group. This soluble substance is formed not only on artificial growth *in vitro* but also during natural infection in man. In patients suffering from lobar pneumonia this specific substance can sometimes be demonstrated in the blood. It is, however, frequently present in the urine in easily demonstrable amounts. The quantity of this substance present in the urine varies with the intensity of the infection and in many instances furnishes a basis for estimating the severity of the disease. In Table VI are presented the relative frequency of occurrence of the specific precipitin reaction in the urine during lobar pneumonia and the outcome of the disease in the cases studied.

TABLE VI.

Specific Precipitin Reaction in Urine in Lobar Pneumonia.

Type of pneumococcus.	Incidence.			Mortality.					
	No. of cases positive.	No. of cases negative.	Positive.	Cases showing positive reactions.			Cases showing negative reactions.		
				No.	Died.		No.	Died.	
			per cent			*per cent*			*per cent*
I	20	15	57.1	20	2	10.0	15	0	0
II	20	8	71.4	20	10	50.0	8	0	0
III	12	5	70.5	12	7	58.3	5	1	20

The urine from 80 cases of pneumonia due to Pneumococcus Types I, II, and III was studied. In 28 of these the urine showed no reaction, and in 52, or 65 per cent, the test was positive. The mortality among the positive Type I cases was low, probably due to the fact that all these cases were treated with specific antipneumococcic serum. Of the 32 cases of Types II and III infection giving a positive urine reaction, 17 died, a mortality of 53.1 per cent. Of 13 cases due to Pneumococcus Types II and III having a negative reaction in the urine, only one died, a mortality of 7.7 per cent.

Most of the cases which fail to show the precipitable substance in the urine recover, whereas the mortality is high among cases in which its presence is demonstrable. The presence of specifically precipitable substance in the urine during lobar pneumonia therefore seems to indicate a severe infection. If the amount present in the

urine increases from day to day, the outcome is usually fatal unless this result is prevented by the administration of antipneumococcic serum.

Significance of Pneumococcus Septicemia.

The suggestion has been made that lobar pneumonia is primarily a blood infection, and that the local process in the lung is but part of a generalized condition. This view has arisen from the discovery that pneumococcus may at times appear in the circulating blood of persons suffering from lobar pneumonia. Reports in recent years have varied considerably as to the frequency with which it is possible to obtain pneumococcus from the blood. Some observers have obtained positive cultures in from 20 to 50 per cent of cases examined and, since these were the more severe cases, they think the presence of pneumococcus in the blood is of bad prognostic import. Others, claiming more suitable technique, have found pneumococcus in the blood during lobar pneumonia with great constancy and attach very little prognostic significance to its presence. In the cases of lobar pneumonia studied in the Hospital of The Rockefeller Institute, pneumococcus has not been found in the blood in all instances. In those cases in which a positive culture was obtained, the course of the infection has been invariably severe and in many instances the disease terminated fatally.

In Table VII are presented the results of blood cultures in a series of 448 cases of lobar pneumonia at the Hospital of The Rockefeller Institute.

TABLE VII.

Relation of Positive Blood Cultures to Mortality in Pneumonia.

Type of pneumococcus.	No. of cases examined.	Blood cultures.				Mortality.			
		Positive.		Negative.		Cases with positive blood culture.		Cases with negative blood culture.	
			per cent		*per cent*		*per cent*		*per cent*
I	145	50	34.5	95	65.5	13	26.0	3	3.1
II	148	49	33.1	99	66.9	36	73.4	9	9.0
III	55	16	29.0	39	71.0	16	100.0	11	28.2
IV	100	21	21.0	79	79.0	11	52.3	3	3.8
Total.....	448	136	30.3	312	69.7	76	55.8	26	8.3

Examination of Table VII shows that of 448 cases of lobar pneumonia, pneumococcus was obtained from the blood in 136 instances, or 30.3 per cent. In spite of the apparently low percentage of positive cultures in comparison with the statistics of some investigators, we feel that this percentage is representative of the number of positive blood cultures obtained by the ordinary hospital routine of taking one or two blood cultures in each case of pneumonia. The taking of repeated blood cultures during the course of the disease would probably raise this percentage. In 37 of these cases in which blood cultures were made at frequent intervals, positive findings were obtained in 50 per cent.

In the 136 cases with positive blood culture the mortality was 55.8 per cent, whereas in 312 cases with negative blood culture the mortality was but 8.3 per cent. These figures seem to show that the presence of pneumococcus in the blood during lobar pneumonia indicates a severe infection and is of bad prognostic significance.

The type of pneumococcus obtained from the blood has an important bearing on the outcome of the disease. In 50 cases in which Type I pneumococcus was obtained from the blood, the mortality was 26 per cent. The fact that this figure is so low is undoubtedly dependent upon the fact that a large number of these cases was treated with Antipneumococcic Serum Type I. Of 49 cases in which Type II pneumococcus was obtained from the blood, the mortality was 73.4 per cent. Of 16 cases in which Type III pneumococcus was recovered from the blood, the mortality was 100 per cent. Of 21 cases in which Type IV pneumococcus was found in the blood, the mortality was 52.3 per cent. These figures indicate that, with the exception of infections with Type I pneumococcus, in which case specific serum is efficacious, the more virulent the type of pneumococcus obtained from the blood, the less is the likelihood of recovery.

Our experience has been that the greater the number of colonies of pneumococcus that develop from 1 cc. of blood when plate cultures are made, the more likely the disease is to have a fatal termination. The following figures indicate the significance of increasing numbers of pneumococci per cc. of blood. The illustration is drawn from our experience with plate cultures made from the blood of individuals with Type II pneumococcus infections. Of 14 cases in which the

numbers of colonies were from 1 to 15 per cc. of blood, 6 died, a mortality of 43 per cent; of 2 cases with 15 to 30 colonies per cc., 2 died; of 4 cases with 30 to 75 colonies per cc., 4 died; of 16 cases with more than 75 per cc., 16 died. It is thus seen that all cases of Type II infection in which the number of colonies per cc. of blood was 15 or more were fatal. This figure, of course, is not to be considered as absolute, but serves to show that cases of Type II infection with considerable numbers of pneumococci per cc. of blood are almost invariably fatal. In some cases of lobar pneumonia, invasion of the blood may occur as late as the 5th or 6th day. A number of these cases are rapidly fatal and study of the blood has shown that the numbers of pneumococci per cc. of blood increased with extraordinary rapidity, the number of colonies in many instances running up into the thousands.

The study of these results leaves little doubt that invasion of the blood by pneumococci in any type of lobar pneumonia is a serious matter, that the seriousness of this invasion is proportional to the virulence of the type of organism, and that the greater the number of organisms present, the more likely is the disease to have a fatal outcome.

Technique of Blood Culture.—Under aseptic precautions 20 cc. of blood may be easily aspirated into a sterile Luer syringe from a convenient vein, usually near the bend of the elbow of the patient. The syringe may be sterilized by boiling for 20 minutes in a covered receptacle, or sterilization may be accomplished by dry heat or the autoclave, the syringe being enclosed in a large cotton-plugged test-tube. The latter method has the advantage of immediate availability when cultures are to be taken. The needles used are preferably nickeled steel needles of about No. 18 gauge. The bevelled points should be sharpened each time before using, to insure ease in penetrating the vein and to reduce the discomfort of the patient to a minimum. With nervous individuals or in those cases where small veins or adipose tissue give promise of difficulty, the skin may be infiltrated with a little 1 per cent novocaine solution. Then, if any trouble is experienced in piercing the vein, the procedure will at least be painless.

When the blood is obtained 10 cc. are added to a flask of 150 cc. of

plain beef infusion broth (page 19) previously warmed to body temperature, and immediately incubated. The remainder of the blood is added in varying amounts to melted plain agar cooled down to about 45°C., and plates are made. Usually after 18 to 24 hours' incubation at 37°C., if the broth culture is positive, the supernatant fluid becomes diffusely turbid, and, on shaking, the mixture appears brownish red to chocolate in color. If very few organisms are present, several days may elapse before any change in the color of the cultures takes place. Consequently broth cultures should be incubated for 7 days and subcultures made on blood agar slants before they are considered sterile.

Colonies on the agar plates usually appear as small white dots, surrounded frequently by a greenish black area due to methemoglobin formation. Identification of the organisms obtained from the broth and agar cultures should be made by the methods described previously (page 29).

Leukocytosis.

It is well known that lobar pneumonia is usually accompanied by an increase in the total number of leukocytes in the blood, as well as by a relative and total increase in the percentage of polymorphonuclear cells. When such a leukocytosis is absent, it may mean a relatively mild infection or an overwhelming one. Chatard (1), in an analysis of 582 cases of pneumonia at the Johns Hopkins Hospital, found a mortality of 55 to 60 per cent in 39 cases having a leukocyte count under 10,000. With the rise in the number of leukocytes from 10,000 to 30,000 there was a progressive decrease in the mortality. Similar experience has been derived from observations of the leukocytes in 463 cases of lobar pneumonia in adults studied at the Hospital of The Rockefeller Institute. Table VIII shows the same striking increased mortality where the leukocytes were below 10,000 per cc., as found by Chatard, and a similar progressive fall in mortality with increasing leukocytosis.

While a single observation of white blood cells may furnish information of prognostic value, a study of the variation in the number and character of the leukocytes during the course of the disease has revealed many points of interest. It has been noted that

TABLE VIII.

The Relation of Leukocytes to Mortality in Lobar Pneumonia.

Leukocytes.	No. of cases.	Mortality.
		per cent
Under 10,000	29	65.5
10,000–20,000	143	23.7
20,000–30,000	177	18.0
30,000–40,000	76	14.4
40,000–50,000	29	24.1
Above 50,000	9	11 0
Total	463	

when consolidation of the lobe or lobes is taking place or during the period when a spread or infection of a new area of the lung is in progress, there may be a well marked decrease in the number of leukocytes. The following observations made on a patient having a Type I pneumococcus infection are illustrative:

Day of disease.	Leukocyte count.	Polymorpho-nuclears.	Lymphocytes.	Large mono-nuclears.	Temperature.
		per cent	*per cent*	*per cent*	°F.
2	15,600	97	3		105
3	13,700	89	8	3	103.6
4	11,200	94	4	2	103.4
5	8,100	88	7	5	103.6
Lesion spreading from lower to upper lobe.					
6	9,200	78	5	17	103
7	14,600	89	5	6	103.8
8	12,600	88	5	7	103.4
10	16,400	78	6	16	103
11	13,200	85	10	5	100
12	17,200	72	15	13	99.8
13	9,200	79	3	17	100.4
14	8,800	79	11	10	100.5

In this case a single observation made on the 5th and 6th days of the disease during the spread of the lesion from the lower to the upper lobe might have given one a false impression of the severity of the process.

A steady rise in the leukocyte count during the course of the disease is usually of favorable import. The following case illustrates this point:

Day of disease.	Leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Eosinophils.	Temperature.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	°F.
4	9,400	93	4	3			103.6
5	9,400	96	4				
	13,600	87	4	7	2		103.6
6	14,400	88	5	5	2		103.2
	14,500	86	13		1		103.6
7	18,700	94	6				101.4
	20,000	86	4	5	5		100.2
8	14,400	84	6	5	4	1	99.2
	12,000	85	3	6	2	4	100
10	9,000	78	6		13	3	98.8
12	8,800	68	10	16	4	2	99.2

On the other hand, if an initial low leukocyte count shows little or no tendency to rise even with specific treatment, the prognosis may be grave. This is illustrated by the following case:

Day of disease.	Leukocyte count.	Temperature.
		°F.
3	8,000	102.5
4	7,200	102.8
	8,800	102.8
5	11,900	102.5
6	12,300	102
7	Died.	

With the fall in temperature by crisis or lysis, the number of leukocytes in the blood rapidly falls in the uncomplicated cases. A continuance of the high count, especially if accompanied by a continued high percentage of polymorphonuclear cells, suggests a complication with pus formation, most frequently empyema or otitis media. A continued leukocytosis with diminishing polymorphonuclear cells and corresponding rise in lymphocytic cells, with the presence of a few eosinophils, suggests delayed resolution, or serum sickness if the case has been treated with specific serum.

The appearance and staining qualities of the white blood cells often reflect the condition of the patient. In those mortally infected, in addition to an abnormally small number of leukocytes, there may be a predominance of degenerated polymorphonuclear cells. The nuclei of these cells appear fragmented, stain poorly, and the cytoplasm presents an appearance suggesting cloudy swelling.

On the other hand, a day or two before crisis, together with the increase in the total number of cells, there may appear many polymorphonuclear cells which may be termed immature forms. The nucleus is large and succulent in appearance and contains large amounts of chromatin material. The cytoplasm of the cell is, in addition, packed with well staining coarse granules. These minor changes that take place in the white blood cells in the presence of an infection, while of less practical value from the standpoint of prognosis than the information that may be derived from the determination of the type of infecting organism, the presence or absence of precipitable substance in the urine, or the occurrence of pneumococcus in the blood, are nevertheless of considerable importance.

ANTIPNEUMOCOCCIC SERUM.

It was demonstrated as early as 1891 that animals such as the rabbit can be rendered immune to lethal doses of pneumococci by the previous injection of sublethal doses of the organisms or by the injection of dead cultures. Furthermore, it was shown that the serum of these immune animals is able to protect other animals against a fatal infection, and even to cure them after infection has once occurred, provided that the injection of the serum is not too long delayed. It is not surprising that attempts were at once made to apply these fundamental facts to the treatment of lobar pneumonia in man. The serum of horses immunized to pneumococci has been widely employed in treatment (17, 20, 25, 26, 32, 33), but the results obtained have been inconclusive, and a few years ago the concensus of the best clinical opinion was that antipneumococcic serum was of little or no value.

It must be remembered that most of these efforts to treat pneumonia with immune serum were made before we had any knowledge concerning the immunologic differences between various races of pneumococci; they were all considered identical. Moreover, at the time these attempts were made to employ immune serum, there were no methods for accurate etiologic diagnosis in the individual cases, and the serum was administered to all cases indiscriminately. It is little wonder, therefore, that the results of treatment were unsatisfactory and inconclusive. Moreover, the immune sera were employed in small amounts and were injected subcutaneously.

We now know from experiments on animals that an antipneumococcic serum is only protective and curative provided it is employed to combat an infection due to the same type of organism as that used in its production. We also know that to cure after infection has once occurred, the serum must be employed in very large amounts, and preferably injected intravenously.

The discovery of these facts, together with the development of methods for making an etiologic diagnosis in every case, made it

advisable to undertake again the treatment of cases of pneumonia with the serum of horses immunized to the various specific types. These attempts have resulted in the demonstration that antipneumococcic serum, prepared by the injection of Type I pneumococcus, is highly effective in the treatment of cases of pneumonia due to the same type of organism. The serum of Type II is much less efficacious. Indeed, it has not yet been thoroughly demonstrated whether it has any practical effect on the outcome of the disease or not. Immune serum may be prepared by the injection of Type III pneumococci and this serum has slight agglutinating power and slight protective power for animals (30). This power, however, is so slight that the therapeutic application of this serum has not been considered promising or justifiable. From what has previously been stated it would be impossible to prepare a serum which would be effective against any considerable number of cases of infection due to Type IV pneumococci.

Production of Serum.

In spite of all that has been written concerning the theoretic principles involved in the preparation of antipneumococcic serum, and in spite of all the reports of the therapeutic application of various sera which have appeared, very little has been written concerning the actual method of preparation of these sera. In general, they have been prepared by the injection into horses at weekly intervals of gradually increasing doses, first of dead, then of living pneumococci.

Based on certain experimental studies which have been carried on in the Hospital of The Rockefeller Institute, this method of producing the serum has been modified, so that the time required has been materially lessened (R31).

What is attempted in the production of immune serum is to obtain, as rapidly as possible, a serum possessing the highest possible content of the known antibodies, agglutinins and bacteriotropic substances, and the highest possible protective action against pneumococcal infection, as tested in mice. Whether such a serum meets all the theoretic requirements is not at present our concern. Moreover, this serum should be specific; that is, effective against the type of pneumococcus causing the infection. Up to the present time antipneumo-

coccic serum has been demonstrated to be effective only in cases due to Type I pneumococcus, so in our opinion the production of antipneumococcic serum for routine practical use should, for the present, be confined to that prepared by the injection of this type of pneumococcus. At the present time the production of a polyvalent antipneumococcic serum is not practical or important, as it is in the case of the production of polyvalent meningococcic serum, for instance. The conditions as regards specificity among meningococci are quite different from those obtaining among pneumococci. The former organisms also differ among themselves in their immunity reactions. However, they all possess certain common characters, so that an immune serum against any one type has some action against all meningococci. With pneumococci, at least as regards the first three types, the immunity reactions are almost absolutely specific. A Type I serum is hardly more effective against infection due to Type II pneumococci than antidiphtheria serum would be. For these reasons we do not advise the routine manufacture of polyvalent antipneumococcic serum.

Concerning the size and spacing of doses, we have found from numerous experiments in rabbits that instead of the old method of weekly injections, much better results are obtained if the injections of dead organisms are made daily for a period of 7 days; then after another period of 7 days elapses, a similar series of injections is made. It has been proved that the doses should be small, as very large doses inhibit the immunity response. After several series of injections of dead culture, it may be necessary to employ the live bacteria. Here it is well to take advantage of the observations of Fornet and Müller, which have already been used by Flexner and his assistants in the manufacture of antimeningitic and antidysenteric serum. In this method the injections are made daily over a period of 3 days, determining the size of the dose largely by the reaction obtained. The following is a brief statement of the method of immunization as actually employed by us at present:

Technique of Immunizing Animals.—A sound, fairly heavy horse should be chosen and quarantined until a glanders test is performed. A specimen of blood obtained before any treatment is given is kept as a control. The immunization is carried on as follows: All in-

jections are made intravenously, employing for this purpose a Luer syringe. To avoid any accidental injury to the vein it is well to have the needle attached to the syringe with a small piece of rubber tubing. The strain of pneumococcus (living or dead) which is used for injection should be one highly virulent for mice (0.000001 cc. kills regularly) and it should have had very few passages either through animals or in artificial medium since removal from the human patient. The method for keeping these cultures is described above (page 13). In preparing the material for injection, both living and dead organisms, cultures are grown on beef peptone broth, reaction 0.3 to 0.5 per cent acid to phenolphthalein. Cultures about 12 to 15 hours old are preferable, as at this time maximum growth is present, with a minimum of autolysis. The cultures should contain about 200 to 300 million bacteria per cc. The culture is centrifuged until the supernatant fluid is clear. With the large centrifuge employed by us this requires about 20 minutes to an hour. The supernatant fluid is then pipetted off and the sediment is taken up in a small amount of sterile salt solution.

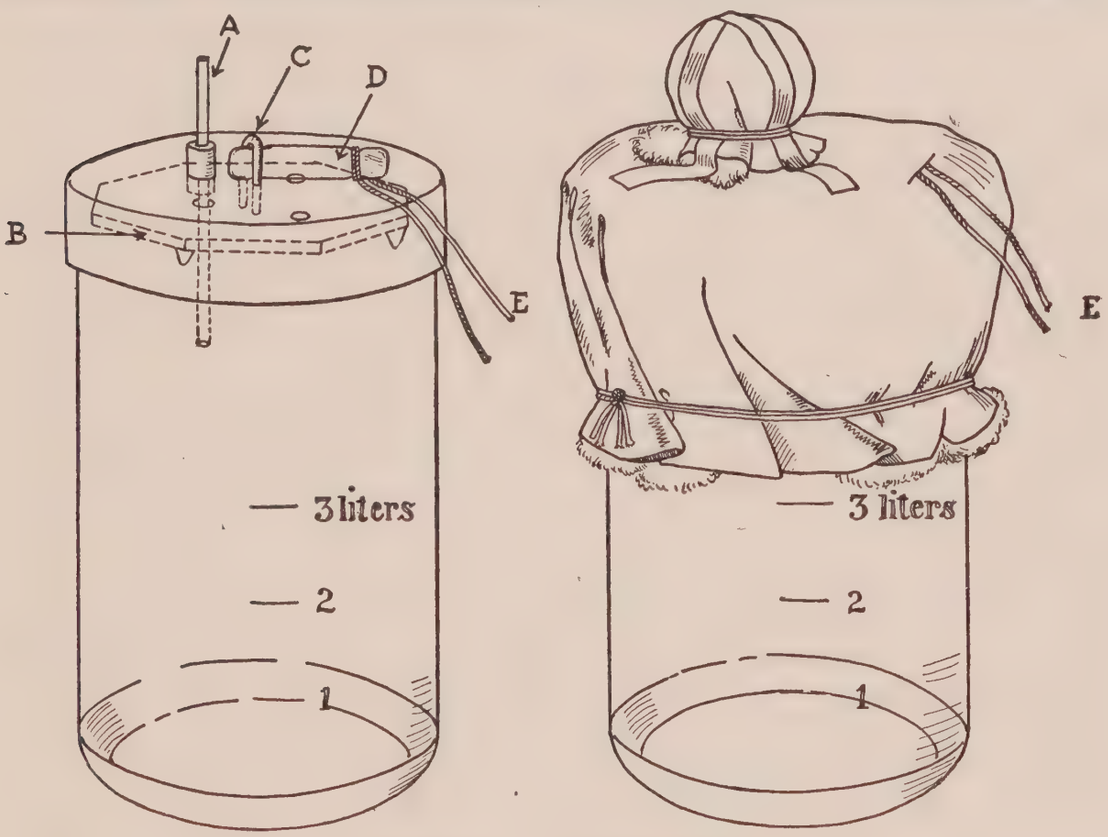
If living organisms are to be injected, the emulsion in salt solution is not made until just before injection, so that death and autolysis may not occur. If the organisms are to be injected dead, the emulsion is heated in a water bath at 56°C. for half an hour. For the daily injections a considerable amount of the vaccine is prepared as above and kept on ice. This vaccine may be employed for all the injections in one series of 6 to 7 days. We think, however, that a fresh emulsion should be prepared for each new series. Of course the emulsion of live bacteria should be freshly prepared for each injection. The dilution of the injected fluid should be such that the total volume is about 20 cc.

The following course of injections is now carried out: Every day for 6 days an injection of the emulsion containing bacteria from 50 cc. of bouillon culture is made. An interval of 7 days is allowed to elapse and then a second series of daily injections of the same sized doses is made. Again an interval is allowed to elapse and on the 6th day a specimen of blood is obtained for testing. Tests are made at once for agglutinating and protective power. This requires several days.

If the serum causes agglutination in a dilution of 1:200 and is of standard protective value, that is, 0.2 cc. protects a mouse against 0.1 cc. of virulent culture, bleeding could be carried out at once (10th to 12th day). As a matter of fact, we have never seen the titer of the serum after this amount of treatment to be so high. Consequently it has been our practice, 8 to 10 days after the last injection of the second series of dead cultures, to commence injections of living bacteria. Three injections are given on successive days. The temperature of the horse is taken every 2 hours for 8 to 10 hours following each of the injections of living bacteria. The first injection should contain the bacteria from 2.5 cc. of the original culture. If the temperature reaction following this injection is only moderate, not over 40.5°C., a second injection of the bacteria from 5 cc. growth is given on the following day. If the reaction from this is only moderate, the dose is again doubled on the following day, the bacteria from 10 cc. of bouillon being injected. It must be stated that as far as pneumococci are concerned it is difficult to regulate the dosage entirely by the febrile reaction obtained. If the reaction is very severe, of course the dose is made smaller than that mentioned. On the other hand, even though the reaction is very slight, we do not advise giving more than the amounts stated. 6 days after the last injection another specimen of blood is obtained for testing. If the serum is of the standard strength, bleeding can now be done. If, however, the potency is still too low, a second series of injections of living bacteria is made. These injections, unless there are reasons for modifying them, should consist of the bacteria from 20, 40, and 80 cc. of broth on the 3 days respectively. Again, the size of the doses may need to be somewhat modified, on account of the severity, or the lack, of febrile reaction. Most horses will require still further injections of living cultures. In any case, we think they should be made in series of three, given on successive days, with 7 day intervals between the series. The third series in most horses may consist of the bacteria from 100, 150 and 200 cc. of broth. From our present standpoint we believe that the size of an injection should never exceed the bacteria from 300 to 400 cc. of broth. Following the bleeding, it is well to allow the horse to remain quiet for 3 or 4 days. Then a series of three injections of living cultures, 50, 80, and 100 cc., is again given. After a week the serum is again

tested, and if of standard strength, bleeding may be done on the 10th day. If the serum is not sufficiently strong, a second series of injections of living bacteria is given, again keeping the dosage fairly small, never giving more than the bacteria from 300 to 400 cc., and always bleeding about 10 days after the last injection.

Technique of Obtaining Serum.—The bleeding of the horse usually takes place about 10 days after the last injection of antigen. It is possibly well to allow the horse to fast 12 to 18 hours before bleeding. The horse is then brushed thoroughly



TEXT-FIG. 1. Bleeding jars.

and its coat washed down with a dilute lysol solution. The floor, walls, and ceiling of the bleeding room are also washed down. The neck of the horse over the external jugular vein is shaved, washed with alcohol, painted with tincture of iodine, and anesthetized with a sterile 2 per cent solution of novocaine. The skin over the vein is incised and the vein is punctured with a cannula bearing a rubber tube, through which the blood is drawn into the receiving jar. This cannula is 8 cm. in length and 5 mm. in bore; the end of the cannula is bevelled for 1.5 cm., has a sharp point, and the bevel is turned against the blood stream. The bleeding jar (Text-fig. 1) is of glass, cylindric in shape, open at the top, 28 cm. in height, and 16 cm. in diameter, a slight modification of the type of jar

described by Kraus and Levaditi (8); we have found "Nonex" glass very satisfactory. The jar bears a loosely fitting cover of tinned copper having an eccentric and a concentric opening; the former carries an ordinary cork through which passes the glass, inflow tube (*A*), to which is connected the rubber tube from the cannula; the inflow tube reaches about 1 inch below the cover. Suspended from the cover, when it is in position on the jar, is a flat octangular weight (*B*) of tinned copper, with an aperture through which the inflow tube passes. This weight is 14.5 cm. in diameter and weighs about 700 gm. On the under surface of the weight are four projecting points or teeth, about ½ inch in length, which serve to prevent it from slipping off the clot when dropped. The weight is suspended in the jar by a loop of wire (*C*) which passes through the concentric opening in the cover. By means of a small piece of wood or metal (*D*) passed through this loop, the weight is held in position. This piece of wood acts as a trigger which, when withdrawn, allows the weight to fall on the clot.

When the cover, weight, and inflow tube are in position on the jar, the whole is covered by a layer of paper which is tied around the jar. Through the paper the inflow tube is allowed to project. On top of this, the inflow tube still projecting, is placed a layer of cotton and another layer of paper, and these are tied securely; a cotton cap enclosed in paper is now secured over the inflow tube. A cord (*E*) attached to one end of the wooden support of the weight comes to the exterior through the paper and cotton covers. The jar so prepared is sterilized in the autoclave at 15 pounds' pressure for 20 minutes and afterwards dried at 100°C. by dry heat. It is advisable to place a few cc. of water in the jar before placing the latter in the autoclave.

Each jar is graduated and usually 3.5 liters of blood are drawn into each one. The cannula and tube are sterilized by boiling for 20 minutes. The cannula should have a sharp, cutting edge, which should be protected during sterilization by wrapping the instrument in cotton. When the cannula is withdrawn from the vein, usually no difficulty is experienced in stopping the blood flow, pressure and perhaps a stitch in the skin being sufficient in most cases.

6 to 8 hours after the bleeding, when the clot is firm, the trigger supporting the weight is released by pulling the attached cord (*E*); the weight then drops on the clot and materially aids in expressing the serum. The serum is usually drawn off the clot by syphonage about 40 hours after the bleeding, the short limb of the syphon being introduced into the jar through the central opening (now free) in the cover. The serum is syphoned into flasks of 6 liters' capacity. The long limb of the syphon passes into the flask through a doubly perforated rubber cork. Through the other perforation there passes a short cotton-plugged glass tube through which partial exhaustion of the flask starts the syphon action. Care should be taken in the syphonage not to carry over particles of clot or cellular débris. The serum is stored in these 6 liter flasks, sealed with rubber corks, and capped with sterile tin-foil. The syphon and corks are sterilized by boiling and the bottles (plugged with cotton and capped with paper) are sterilized in the

autoclave and afterwards dried at 100°C. by dry heat. Usually at a single bleeding 9 to 12 liters of blood are withdrawn and from this, employing the method described, we have usually obtained a yield of 50 to 55 per cent of serum.

Storage of Serum.—The serum is stored in the refrigerator in the 6 liter bottles for at least 2 months. During this period a fine precipitate gradually sediments. It is desirable that this sedimentation should be complete before the serum is dispensed for treatment in order to avoid the possibility of certain reactions on the part of the patient, due to the intravenous injection of suspended particles.

Dispensing Serum.—After the requisite period of storage, the serum is dispensed for treatment in sterile, rubber-corked bottles of 100 cc. capacity, capped with parchment which has been soaked in 1:20 phenol. This final bottling of the serum is accomplished by syphonage; this time, however, the syphon action is started by raising the air pressure in the storage flask through a cotton-plugged tube in the rubber stopper through which the syphon tube also passes. The syphon tube terminates in a specially designed filling device which, by means of a protecting bell or jacket, covers the neck of the dispensing bottle, thus avoiding unnecessary exposure of the serum.

All the handling of the serum is conducted in a room free from draughts and dust. After bottling, the serum is tested for agglutination and protective power and it is also subjected to a rigid test for sterility and for primary toxicity.

Tests for Sterility. Culture Tests.—The first, middle, and last of the dispensing bottles from each storage flask of serum are set aside for sterility tests. From each of these bottles, 0.5 cc. and 4 cc. of serum are each mixed with about 20 cc. of 1 per cent glucose broth in Smith fermentation tubes layered with paraffin; 2 cc. of serum are mixed with about 15 cc. of melted 1 per cent glucose agar at 43°C. in deep tubes, and, after cooling, layered with paraffin; 6 cc. of the serum are mixed with 150 cc. of ordinary nutrient broth; all are then incubated at 37.5°C., and observed for 14 days. If no growth or gas production is observed in any of the cultures, the particular lot of serum is considered sterile.

Animal Tests.—4 cc. of the serum are injected subcutaneously into each of two guinea pigs weighing 250 to 300 gm.; the guinea pigs are

observed for 2 weeks for evidence of toxicity or infection. 0.5 cc. of serum is also injected into each of two mice which are observed for 4 days. This test is for the purpose of making sure that the serum contains no living pneumococci from the last injection. However, in these tests we have never found pneumococci present.

Use of Antiseptics.—Employing the precautions we have mentioned, it has been rare for any lot or sample of serum to become contaminated. Where large amounts of serum are injected intravenously, as is necessary in this form of treatment, the ideal method is to employ serum to which no preservative has been added, and under the conditions in which we have employed the serum, we have shown that this is practical. The serum should, of course, be kept in the dark and in the cold. Under these conditions serum which we have kept several years has remained uncontaminated. If manufacturers feel that this method on a large scale is impractical, chloroform or tricresol may be added to the serum before bottling.

At present, most State Board of Health laboratories employ chloroform for the preservation of serum which is to be injected intravenously, and this is probably preferable to tricresol or other phenol preparations, though in the treatment of cholera large doses of immune serum, which have contained 0.5 per cent phenol, are said to have been employed (7) without injurious effects. Undoubtedly, however, where large amounts of serum are to be injected, the use of a volatile preservative, as chloroform, is preferable. The following is the method at present employed by the Department of Health of the City of New York: After the serum is drawn into the large flasks, an excess of chloroform is added, about 5 cc. to 1 liter. When the serum is drawn off for bottling, the excess of chloroform is left, together with the sediment, at the bottom of the flask.

Preservation of Potency.—We have examined samples of serum kept on ice and in the dark after a period of 2 years. No loss of strength could be detected by the methods used. If allowed to stand at room temperature and in the light, a fairly rapid loss of potency occurs. In the experience of Neufeld and Händel (14) also, antipneumococcic serum keeps many months unchanged. They also found that neither heating to 59°C. nor the addition of carbolic acid causes any loss in potency.

Concentration of Serum.—Experimental evidence and clinical experience have demonstrated that antipneumococcic serum must be administered in large doses if success is to be obtained in this method of therapy. Any method of concentrating the serum without decreasing its antibacterial potency, therefore, would be of great value in rendering its administration more convenient and practical. If concentrated serum were employed, moreover, the frequency of serum disease would be decreased, or at least the symptoms would be ameliorated.

Study of the distribution of the immune bodies occurring in antipneumococcic serum has shown that both the agglutinins and protective antibodies are associated or combined with the globulins, and not with the albumin of the serum (R2). Hence concentration by fractional precipitation of the globulin fraction with ammonium sulfate is possible. This procedure, however, is attended with certain disadvantages. Bacterial contamination of the serum necessarily occurs during the procedures attending the chemical manipulations. Although the bacteria may be removed by filtration through a Berkefeld filter and their growth inhibited by the addition of preservatives, the products of bacterial metabolism remain and may exhibit a toxic action when injected intravenously. It is doubtful, therefore, whether serum concentrated in this manner should be employed for intravenous use.

Gay and Chickering (R15, R16) have shown that the mixture of a solution of pneumococcus bodies and homologous antiserum results in the formation of a voluminous precipitate, which contains practically all the immune substances of the serum. The immune bodies contained in this precipitate can now be extracted in a dilute alkaline solution at 42°C. The resulting water-clear extract possesses the power to protect animals against pneumococcus infection and it also contains other demonstrable antibodies, such as agglutinins and precipitins. By this method a large proportion of the antibodies can be concentrated in a volume which is only one-fifth to one-tenth that of the original serum, and in which there is only one-sixtieth of the native protein. This biologic method of concentrating antipneumococcic serum, while yielding an end-product which is ideal in many ways, is nevertheless laborious in technique, and the preparations obtained are of very inconstant strength and are not stable.

While, therefore, concentration of antipneumococcic serum can be effected by either chemical or biologic methods, the disadvantages attending both of these processes are such that as yet neither has been found of practical utility.

Standardization of Serum.

In order that any serum may be used therapeutically, it is important that there should be some method of determining the strength or potency of the samples employed. Without this, no constant dosage can be obtained, and any estimate of the effectiveness of the serum is difficult or impossible. The method used for standardization of antitoxic sera, such as diphtheria and tetanus antitoxin, is almost ideal. Here it is possible to establish toxic and antitoxic units, and the law of multiple proportions holds good for these units within very wide limits; if 1 cc. of serum neutralizes 1,000 units of toxin, 0.001 cc. will neutralize 1 unit of toxin, or 100 cc. will neutralize 100,000 units, almost with the constancy of chemical acid-base neutralization.

An analogous method of standardization for antipneumococcic serum has been proposed, but there are great difficulties in the way of using such a method for this serum. No pneumococcus toxin has yet been demonstrated and the employment of an antitoxic unit is therefore impossible. Nevertheless, it might be possible to make use of the same principle of standardization, using bacteria themselves instead of their toxin, provided that a minimal lethal dose of bacteria could be established, and provided that the reaction between the minimal lethal doses and varying quantities of serum obeyed the law of multiple proportions. The attempt to employ such a method has been made by Eyre and Washbourn (3) and others. The establishment of a minimal lethal dose of pneumococci, however, is a matter of extreme difficulty. Pneumococci vary greatly in their virulence from time to time. With every passage through an animal or on artificial culture medium, an alteration occurs. This change may not appear very great, as estimated by our usual methods, but in relation to the great accuracy required in the establishment of a minimal lethal dose, the changes are enormous. It is only in organisms possessing the maximum degree of virulence that the virulence is at all stable. However, when cultures of high virulence are inoculated

into susceptible animals, as rabbits or mice, which are usually employed for these tests, it is found that very few bacteria, at times even a single organism, are sufficient to cause a fatal infection. With these bacteria the determination of the minimal lethal dose would be extremely difficult, if not impossible. Our studies indicate, however, that if such a unit could be established, the antiserum, when tested against it, would probably be found to obey the law of multiple proportions. Nevertheless, on account of the difficulty of accurately establishing a minimal lethal dose, this method does not at the present time seem practicable.

This failure to establish a unit for antipneumococcic serum, however, is not so serious as it might seem. The main practical object to be gained by the employment of this method of standardization would be that the dosage might be very accurately regulated, and that sera of weak power might be employed, provided that a sufficient amount were used. However, experience has shown that the maximum strength of any antipneumococcic serum that can be produced by present methods is limited. This limit is quite constant and differs for the different types of pneumococci employed. In order to obtain practical results in treatment, even with serum of this maximum strength, very large amounts must be used. Therefore, even if we had such a method of standardization as that above mentioned, it would not be practical to employ sera of low potency, nor would it be of great advantage to be able to estimate very accurately the exact potency of the dosage. Such a method of standardization of antipneumococcic serum therefore is, at the present time, neither possible nor necessary.

In the absence of an absolute standard, it might be possible to devise a test for determining the relative value of different sera, by making use of certain phenomena, as agglutination or opsonification. In these phenomena, if the amount of bacteria employed is not too great, the reaction is entirely proportional to the concentration of the serum in the mixture, not to its actual amount. At least, the only method we have at present for measuring the power of a serum to produce agglutination, consists in the determination of the maximum dilution at which the serum acts, and this is practically independent of the number of bacteria acted upon. There-

fore, if the effectiveness of a given serum depended upon its agglutinating power or were proportional to it, this property could be employed to fix the strength of any serum, and in such a method the great variable, the living bacteria, would be eliminated. Even in the absence of an absolute standard, we should then have a relative standard of efficiency. If bacteriotropic power were employed, however, the bacterial variable would not be eliminated, since it has been shown that different bacteria vary greatly in their susceptibility to the action of bacteriotropic sera, and this variation is to some degree dependent upon virulence. In any case, however, the hope of devising a method of standardization, employing either agglutination or bacteriotropic action, has been destroyed by the observation that, while there is frequently some gross quantitative relationship between the protective action of a serum and its agglutinating or bacteriotropic power, this relationship is inconstant. For instance, we have had sera with high protective power and little or no agglutinating power, and *vice versa*.

Although it is not possible to establish an anti-infection unit and although it is not possible to employ the properties of agglutination or opsonification in the ways we have mentioned for estimating the relative strength of sera, it is still possible to have a standard that will be of great practical value in the therapeutic employment of this serum. If a minimum potency for those sera that are to be employed therapeutically can be established, which minimum is not far below the maximum possible, we shall have gone far in standardizing the treatment of cases by serum, even though this method does not make possible fine distinctions in dosage or the use of sera of weak strength. It is, of course, necessary that serum of the strength determined upon can be produced without too great difficulty. Otherwise great waste would result and the whole method would become impractical. In view of what we have already stated in regard to agglutination and opsonification, these properties cannot be employed, but the potency of a given serum must be ascertained by direct determination of the protective power of the serum for animals. In order to establish by this method such a limit of potency as we have suggested above, there must be some constant relationship between the size of the infection dose and the amount of serum necessary to

protect; in other words, the serum must obey the law of multiple proportions.

Neufeld (14), who first studied this question, decided that antipneumococcic serum, as tested by protective power, obeyed this law only within given limits and under certain conditions. His experiments seemed to show that the results were regular until the serum had been diminished to a certain amount, depending on the weight of the animal employed. When an amount of serum less than this was employed, the relation of serum to culture no longer held constant; this smaller amount of serum would protect only against a very much less amount of culture than that calculated. From this he judged that to obtain effective action, even against minimal doses of culture, a given concentration of serum in the body (not actual amount) is required. This effective limit to the action of serum he called the *Schwellenwert*. His experiments were performed on mice and he did not mix the culture and serum before injection, but injected them separately in different parts of the body and usually allowed a period of time to elapse between the injection of the serum and that of the culture.

This matter was later studied in this hospital, but rats were employed and the given amounts of culture and serum were mixed in the syringe immediately before injection. It was found that the law of multiple proportions held valid as long as small amounts of culture and serum were employed; that even extraordinarily small amounts of serum would protect against proportionally small doses of culture. The relative amount of serum necessary to protect against larger doses of culture became greater and greater, however, as the size of the doses of culture was increased, until finally no amount of serum, however large, was able to protect.

The explanation of Neufeld's results lies in the fact that the serum was injected separately from the culture and, owing to the length of time required for diffusion of small amounts, multiplication of the bacteria had occurred and the degree of infection had increased enormously and the infection had become widespread before the serum became effective at the site of injection. Our experiments suggest that in the action of immune serum a body or tissue factor is essential, in addition to the substance contained in the serum. If the infection is not too great, this factor is adequate. If, however, the infection is enormous, this factor is no longer sufficient. Within given limits, however, any variations in this factor are not important and, moreover, within these limits the relation of serum to culture is fairly

constant. This fact is of great importance, as upon its validity depends the possibility of establishing a standard of potency.

From these experiments and those of Neufeld, it has been decided justifiable to fix a definite standard of strength to which sera used for therapeutic purposes should conform, determining this strength by direct estimations of the protective action of a definite fixed amount of the serum for mice against variable amounts of culture of high virulence. In such estimations as these it is obvious that while variations in virulence are important, slight variations make no great difference, as they would in determination of the minimal lethal dose, provided that the virulence is near maximum. The amount of serum to be employed in the tests also is not of great importance, provided that it is always the same and provided that it is selected within the limits where the law of multiple proportions holds valid.

Theoretically, it would be better to titrate several amounts of serum against varying amounts of culture, choosing these amounts of serum at various levels. Practically, however, this would render the method too complicated for ordinary use, and it has been found sufficient to use one amount of serum, provided that this amount is not too great or too small, and that the same amount is used for all tests.

Neufeld (17) used 0.2 cc. of serum for this purpose and we have found this satisfactory. Wadsworth and Kirkbride (30) have recommended using 0.1 cc. of serum without, however, giving any definite reasons for the change. Since much work has already been done using 0.2 cc., it seems best to continue this method.

We realize that the method of standardization which we advise is far from ideal. At the present time, however, it seems to be the only practical one, and, if applied with care and attention to details, it will at least prevent the use of sera of low potency, and, we believe, will make the therapeutic use of antipneumococcic serum both possible and effective.

Technique of Standardization Test.—In making a practical test of the protective power of the serum, white mice weighing 18 to 22 gm. are employed. The largest amount of an 18 hour broth culture of the homologous pneumococcus against which a constant quantity of the serum, 0.2 cc., will protect, is determined. Various dilutions of the broth culture are so prepared that each 0.5 cc. of the mixtures

contains a given quantity of the pneumococcus culture, from 0.2 cc. to 0.0000001 cc. The dilutions are made in peptone broth similar to that in which the bacteria are grown, and a separate pipette is used for making each dilution in order that an accurate distribution of the bacteria may be obtained, and to avoid carrying over any bacteria from the lower to the higher dilutions. The dilutions are made immediately before being used so that no growth or other change may occur in the mixtures. At the same time a small amount of the serum is diluted in broth, two parts of serum to three of broth, so that 0.2 cc. of serum is contained in each 0.5 cc. of the mixture. When injecting the animal, 0.5 cc. of the diluted serum is taken up into a 2 cc. Luer syringe and then 0.5 cc. of the diluted culture which is to be injected. The mixture of serum and culture is then injected at once intraperitoneally. The needle of the syringe is run under the skin a little above and parallel to Poupart's ligament and then obliquely through the abdominal wall. By this method injury to the liver, large blood vessels, or kidneys is avoided. In making a test of a given amount of serum, usually injections are made of 0.2, 0.1, 0.01, 0.001, and 0.0001 cc. of culture, each mixed with 0.2 cc. of the serum to be tested. It is best to inject two mice with each of these amounts. In making the series of injections one should commence with the mixtures containing the smallest amount of culture. It is then possible to use one syringe for all the injections. As a control of the virulence of the organisms used, another series of mice should be injected with these dilutions of culture, each with 0.2 cc. of the standard serum. Any marked variations in the virulence of the organism employed will then become evident, since by the protective power of the standard serum against the culture of organism employed, the virulence of the latter may be estimated. As a further control of the virulence of the organism, three mice are injected intraperitoneally, one receiving 0.0000001 cc., one 0.000001 cc., and one 0.00001 cc. of culture. The actual number of organisms contained in the culture employed is determined by making agar plates from each of these dilutions of culture and estimating the number of colonies appearing after 24 hours' incubation. With the same proportion of peptone in the broth and a standard acidity, it is surprising how nearly equal the numbers of bacteria contained in 1 cc. of each of the

various cultures are. Usually 0.0000001 cc. of culture gives rise to 10 to 30 colonies on the plate, which means that the number of bacteria contained in 1 cc. of the broth culture is from 100 to 300 million. It is our plan to mark the mice by staining a given part with carmine, and it is well to have a regular system for doing this and marking each animal before it is released from the hand. As the mice are injected, they are placed in glass jars containing sawdust and covered with pieces of wire netting. Care should be taken that they are not overcrowded (not more than three or four mice in a jar) and they should be kept in a room with an even temperature. They should be observed over a period of 5 days. With a virulent pneumococcus the controls, receiving culture alone, should die within 48 hours. These mice, as well as those unprotected by the serum, should have cultures made from the heart's blood in order to make sure that pneumococci are present and that some extraneous factor has not been the cause of death.

The best Type I sera that we have been able to produce have not protected regularly against doses higher than 0.2 to 0.5 cc. of culture. Most of them have not protected regularly against doses over 0.2 cc. We believe, therefore, that for therapeutic use only those sera should be employed which in doses of 0.2 cc. protect regularly against at least 0.1 cc. of a culture which is shown by the animal and standard serum tests to be of proper virulence. The virulence of the organism used in the protection test is usually such that 0.000001 cc. of an 18 hour broth culture will kill a mouse of 20 gm. weight within 48 hours. The following protocol illustrates the exact method of carrying out the test:

Typical Protocol of Standardization Test.

No. of mouse.	Marked.	Culture.	Serum.	Result.
		Serum, Horse XX, Lot 4.		
		cc.	*cc.*	
1	Head.	0.2	0.2	D.* 48 hrs.
2	"	0.2	0.2	S. 5 days.
3	Back.	0.1	0.2	S. 5 "
4	"	0.1	0.2	S. 5 "
5	Right hind leg.	0.01	0.2	S. 5 "
6	" " "	0.01	0.2	S. 5 "
7	Left " "	0.001	0.2	S. 5 "
8	" " "	0.001	0.2	S. 5 "
9	Right fore "	0.0001	0.2	S. 5 "
10	" " "	0.0001	0.2	S. 5 "
		Standard serum.		
11	Head.	0.5	0.2	D. 4 days.
12	Back.	0.4	0.2	D. 48 hrs.
13	Right hind leg.	0.3	0.2	S. 5 days.
14	Left " "	0.2	0.2	S. 5 "
15	Right fore "	0.1	0.2	S. 5 "
16	Left " "	0.01	0.2	S. 5 "
17	Head.	0.00001	—	D. 12 hrs.
18	Back.	0.000001	—	D. 24 "
19	Right hind leg.	0.0000001	—	D. 36 "

* D., died; S., survived.

In our experience sera produced by the injection of Type II organisms never regularly protect higher than in the ratio of 0.2 cc. of serum to 0.01 cc. of culture. The protective power of Type III serum is still less.

SERUM TREATMENT.

Indications for Serum Treatment.

It has been proposed by others that immediately after admission to a hospital or after coming under the care of a physician and before the type of infection is determined, every patient should receive a large dose of antipneumococcic serum of Type I or a polyvalent serum containing antibodies to Type I pneumococcus. The purpose of employing this method is to avoid the delay incident to the determination of the nature of the infection. Later, if the infection proves to be due to Type I pneumococcus, the treatment may be continued; otherwise it may be stopped. As we shall show later, the administration of serum is frequently followed by general constitutional reactions and, if this method were employed, many patients in whom the serum could be of no possible value would be subjected to these reactions. The time gained would not be great, since, in most cases, the type of infection can be determined within 6 to 18 hours, and during this time, if the patient is sensitive to horse serum, he can be desensitized. Pneumonia is the easiest of the acute infectious diseases to diagnose. If the importance of early diagnosis and of type determinations were generally recognized, serum treatment could be commenced very early in most patients, even though the etiologic diagnosis caused slight delay. We therefore believe that except in emergencies the administration of serum should only follow the determination of the nature of the infection.

As soon as the type of pneumococcus responsible for the infection has been determined, if it proves to be Type I, serum treatment should be undertaken at once in all cases, except in very young children who appear but little intoxicated by the infection and in whom the prognosis is good and the difficulty of treatment great. In the case of adults, first seen late in the disease, apparently mildly ill, and already showing signs of decreasing fever and intoxication, serum treatment may also be unnecessary. When cases are seen late

in the disease, still very ill, one should be extremely careful to eliminate complications as the cause of the symptoms. The value of the intravenous use of antipneumococcic serum in otitis media, empyema, pelvic or joint abscesses, pneumococcus pericarditis, or meningeal infections has not been demonstrated. Occasionally one is tempted not to use serum, or at least to wait a day or two, in cases which seem relatively mild at onset. This is believed to be most unwise, as frequently the pathologic process spreads insidiously, giving little evidence of intoxication for several days, when suddenly the picture changes, the pulse, temperature, and respiration rise, cyanosis deepens, and the end comes too soon to be influenced by the tardy use of the specific serum.

Occasionally one first sees a case at a time when consolidation of another lobe is taking place. At such a time the temperature may perhaps be lower and the heart rate and respirations slightly less rapid than previously. On the other hand, the general appearance of the patient does not seem reassuring; he may appear prostrated, and the extremities may be moist and clammy. In a few hours the temperature rises again, and the following day the signs of more extensive consolidation may be more apparent. These cases especially need immediate treatment when first seen if the further spread of the disease is to be prevented.

Administration of Serum.

Antipneumococcic serum can be administered most easily in a hospital where trained assistants and sterile materials are always available, but it may be given anywhere, in the home or in the field, in fact wherever water can be boiled and iodine and alcohol obtained.

Following the administration of foreign serum, especially the large amounts necessary in this form of therapy, the patient may exhibit certain symptoms which are entirely due to the foreign protein and have nothing to do with the content of the serum in antibodies. This reaction is identical with, or at least analogous to the so called anaphylactic reactions seen in animals. It is very important that, before serum is administered, every effort should be made to ascertain

whether patients are highly sensitive to horse serum, in order that such reactions may be guarded against. The detection of serum sensitiveness is not difficult. Every patient with pneumonia, immediately on admission to the hospital or after coming under the physician's care, should receive proper tests to determine this fact, since, if he proves to be infected with Type I pneumococcus, serum should be administered.

The patient is first questioned for a history of previous injections of immune sera for diphtheria, meningitis, or for tetanus infections. He is also questioned concerning previous symptoms suggesting asthma or hay fever, since persons having such symptoms are likely to be sensitive to various proteins, including those in serum. In any event, however, an intradermal skin test is performed.

Intradermal Skin Test.—The skin, preferably over the forearm, is carefully cleaned with soap and water and alcohol, taking care not to make the skin red or inflamed. If the skin is gently patted, instead of rubbed, with a gauze sponge, no irritation develops. With a small syringe, preferably of the type used for tuberculin injections, and a fine needle, about No. 28 gauge, 0.02 cc. of sterile diluted horse serum (normal or immune), diluted with salt solution 1:10, is injected *into* the skin, not subcutaneously, so that a small white wheal about 3 mm. in diameter showing the little depressions of the hair follicles is produced. An injection of a like amount of sterile salt solution is made as a control. It is advisable to make the serum and the control injections at one level on the forearm, about 3 to 4 cm. apart, in order that the test may not be obscured by the possible spreading of the injected material along the lymphatics.

The points of injection should be observed at intervals for 1 hour. Usually after a few minutes the wheal produced by the salt solution disappears, leaving no alteration in the skin except the small needle puncture. If the test is negative, that is, if no evidence is obtained of horse serum sensitiveness, the wheal produced by the serum injection fades away also almost as rapidly. On the other hand, if sensitiveness does exist, usually within 5 minutes a genuine urticarial wheal begins to develop, which may increase slowly in size up to that of a half dollar, the wheal in turn being surrounded by a larger area of erythema. This lesion usually reaches its maximum extent

within 1 hour and fades away rapidly, disappearing entirely within a few hours. A faint erythema may persist for a day or more. There are usually no subjective symptoms from this intradermal injection, except occasionally slight itching at the point of injection. Very rarely, in individuals extremely sensitive to horse serum, the injection of this small amount of serum, 0.002 cc., has been known to produce general symptoms, flushing of the face, increased rapidity of the heart rate, and respiratory discomfort.

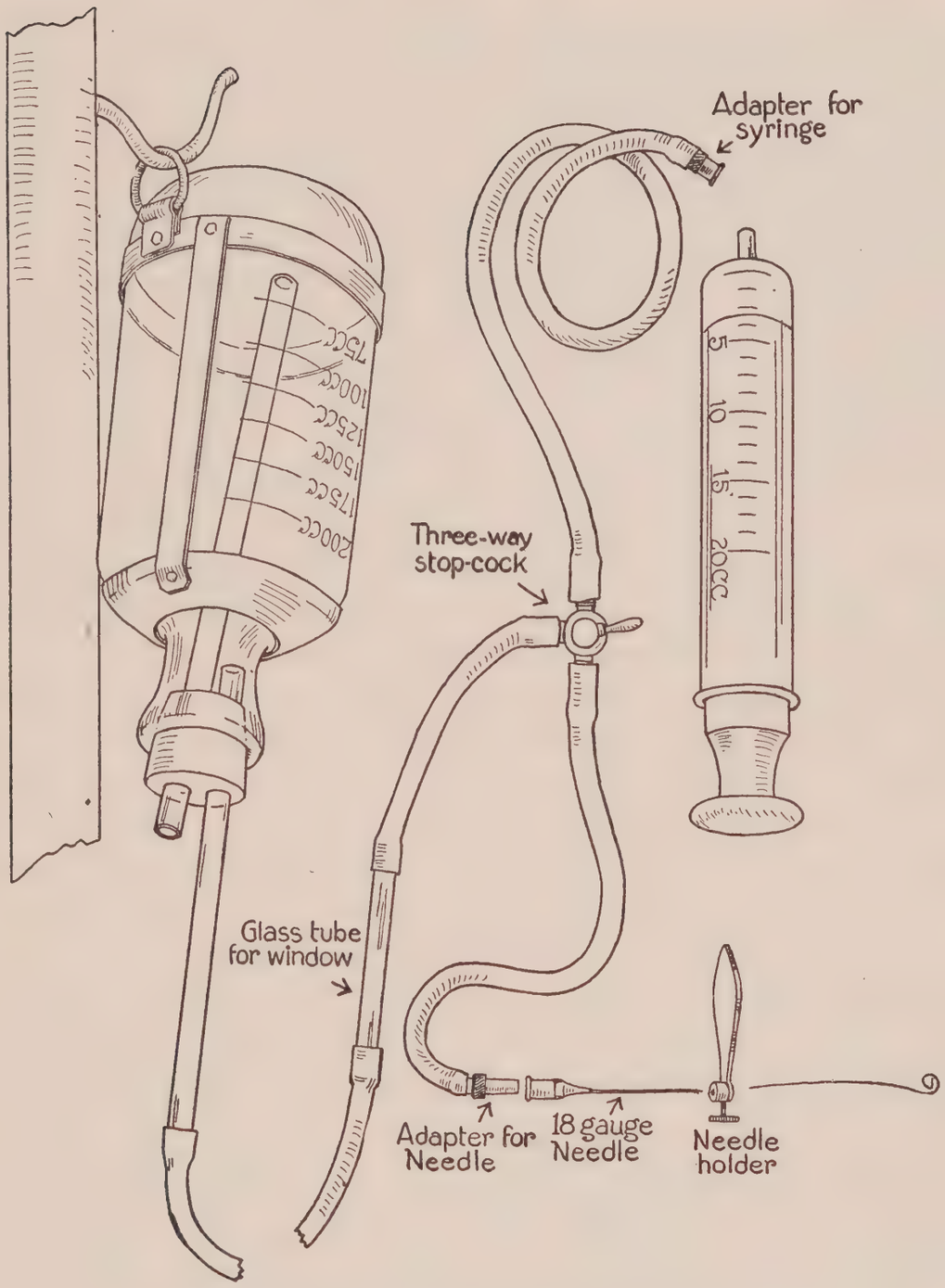
Desensitization.—It has been shown that even very susceptible and highly sensitized animals may be completely desensitized, so that they can tolerate large amounts of the serum to which they are sensitive, if repeated injections are made, beginning with an extremely minute dose and following this with doses of gradually increasing size. Even administering the serum with extreme slowness may be sufficient to prevent anaphylactic shock, or the administration of a very small dose, several hours before the large injection, may protect animals that are not highly sensitive. Therefore, for purposes of desensitization, it is advisable to inject 0.5 to 1 cc. of antipneumococcic serum or sterile normal horse serum subcutaneously into every pneumonia patient as soon as the skin test is completed, even though the intradermal skin test is negative, for occasionally one sees a patient in whom the skin test appears to be negative and yet in whom, on the injection of large amounts of serum, slight symptoms of serum sensitiveness appear. It is believed that the subcutaneous injection of a small dose of serum will desensitize a patient for from 6 to 8 hours to several days. However, this procedure should not be relied upon to the extent of giving the subsequent intravenous dose at a rapid rate. We know of at least one instance in which a desensitizing dose of 1 cc. of serum injected subcutaneously failed to prevent the development of a severe anaphylactic reaction in a patient who had received an intravenous dose of serum 10 days before. In our hands, however, the use of the preliminary intradermal and subcutaneous serum injections, we believe, has contributed much to our freedom from untoward symptoms during or after serum treatment.

If the intradermal skin test is positive, indicating that the patient is sensitive to horse serum and if the infection is due to Type I pneu-

mococcus, in which case the patient should receive serum treatment, a more thorough method for desensitization must be employed. This should consist in giving extremely small amounts of serum subcutaneously at ½ hour intervals, doubling the size of the dose at each injection. One can safely begin with the injection of 0.025 cc. of serum. If no reaction follows the injection of 1 cc., subsequent doses may be given intravenously, also at ½ hour intervals, beginning with 0.1 cc. and doubling the dose at each injection. If a general reaction occurs, or cyanosis, dyspnea, or increased rapidity of the heart rate supervenes, the injections should be suspended for 2 to 4 hours, depending upon the severity of the reaction, and then be resumed, starting with the same dose as that producing the reaction. After 25 cc. of serum have been given in these small doses, after a lapse of 4 hours, 50 cc. may be given, followed by the regular dose 6 to 8 hours later. The desensitization of a patient is a tedious and time-consuming procedure. Fortunately one rarely is called upon to employ this measure. It has been found necessary to desensitize in this way only two or three patients in a series of over 150 cases.

Technique of Treatment.—In preparing for the intravenous injection of serum it is best, though not necessary, to have a sterile field; that is, sterile towels, gloves, etc. It is, however, quite possible to inject serum aseptically with only a package of sterile gauze sponges and iodine and alcohol to disinfect the patient's skin about the point of injection. The apparatus for injecting the serum is shown in Text-fig. 2 and consists of the following:

1. Sterile container for mixture of antipneumococcic serum and sterile freshly distilled normal salt solution.
2. Rubber tubing, 4 feet long, and a three-way stop-cock.
3. Short piece of glass tubing for a window.
4. Rubber stopper with double holes through which are thrust two pieces of glass tubing. One tube is long enough to reach nearly to the bottom of the container, and through this air enters the container when the latter is inverted. The other shorter glass tube forms the outlet for the serum.
5. Two sharp needles and needle holder.
6. One 20 cc. Luer all glass syringe.
7. One 2 cc. Luer all glass syringe with hypodermic needles.
8. Thumb forceps.
9. Medicine glass.



TEXT-FIG. 2. Apparatus for injection of serum.

All this apparatus should be assembled and sterilized by boiling for 20 minutes in a covered dish. It is important to rinse out the tubing thoroughly after it has been used; otherwise with a subsequent sterilization any serum remaining in the tubing will be coagu-

lated and plug the apparatus. This material in the tubing may also be responsible for unexpected reactions on the part of the patient when it is accidentally injected along with the serum. To avoid this difficulty, the rubber tubing may be almost entirely replaced by lengths of glass tubing short enough to be contained in the sterilizing receptacle. These are connected by short pieces of rubber tubing 2 inches long, which are easily cleaned and can frequently be discarded for fresh ones. The serum should be warmed before injection to slightly above body temperature. It has been customary to dilute the antipneumococcic serum with equal parts of sterile freshly distilled (glass still) salt solution just before the injection is made. This makes the serum less viscous and facilitates its flow through the tubing. If salt solution properly prepared for intravenous use cannot be obtained, the serum may be used in its undiluted condition. The slow injection of the smaller amount of fluid during 25 to 30 minutes is, however, somewhat difficult.

When one is ready to make the injection, the serum is transferred to the container by means of a sterile pipette. In this way the transference of the small amount of sediment which is always present in the bottom of the bottle (if care has been taken to prevent shaking) can be avoided. If no pipette is at hand, the serum may be carefully poured from the bottle to the container, taking care that the sediment is not disturbed. This sediment may do no special harm, but its administration should be avoided if possible. When no container is available, the serum may be injected directly from the bottle, with a syringe with a long needle. The method described, however, is the one of choice.

Having filled the container, the cork is inserted and prevented from falling out by strips of adhesive tape. The bottle is inverted and attached to the side of the bed or to an irrigating stand. After allowing the serum to fill the tubing, the needle is introduced into a convenient vein, usually at the bend of the elbow, the overlying skin being previously cleansed with iodine and alcohol. If one has facilities for making blood cultures, it is wise to take about 10 to 15 cc. of blood for blood culture before beginning the serum injection. The serum may be allowed to run into the vein very slowly by gravity, but may be more easily and accurately controlled by aspirating it

into the syringe and then in turn injecting it into the vein (Fig. 5). In this way it is possible, and indeed advisable, that the steady and slow injection of the first 10 to 15 cc. of serum should occupy 10 to 15 minutes. During this period one watches carefully for any change in the patient's appearance and for increased rapidity of the pulse, respiratory difficulty, pallor, cyanosis, or urticaria. If these symptoms appear, it is well to stop the injection of serum for a few minutes, 15 to 20, to see if the symptoms increase in severity. Usually they rapidly disappear and the injection can be resumed. The needle can be easily kept free from clots by slowly injecting salt solution with the detached syringe. The serum can be kept at about body temperature when being injected slowly by placing the tubing between two hot water bags just before it reaches the patient.

Except in very rare instances, no symptoms arise during the injection of the first 10 or 15 cc. of serum, and the injection may then be completed more rapidly in 10 to 15 minutes. As soon as the needle is in the vein and is kept motionless, the patient experiences no discomfort; in fact, in most cases the patient dozes quietly during the treatment. With nervous individuals, the infiltration of a small area of the skin at the point of injection with 1 per cent cocaine or novocaine solution renders the whole procedure painless.

Dosage and Frequency of Treatments.

Practical experience has now shown that for antipneumococcic serum to be effective it must be given in large doses and intravenously, in order that a wide distribution of immune bodies throughout the body may occur rapidly. As previously stated, the exact mode of action of the serum is not yet certain; nevertheless to obtain satisfactory results, it is necessary to inject sufficient serum to produce in the patient's blood a concentration of antibodies such as that which occurs during natural recovery from the disease, for we believe that it is largely upon this factor that recovery depends (15, R32). If to obtain this result required merely the addition of a definite amount of the serum to the patient's blood, just as we might make a dilution of the serum in normal blood in the test-tube, there would be little difficulty. However, experiments have shown that (R

30) not only do the bacteria circulating in the blood fix antibodies and so render them ineffective, but in the infected patients the blood contains soluble substances which fix antibodies just as do the bacteria themselves. In the severely infected patients these soluble substances may be present in very large amounts, and it is only after these substances are all saturated that an effective concentration of immune bodies in the blood can be obtained. Moreover, it is not only necessary that the desired concentration of antibodies should be present immediately following the injection, but also that this concentration should be maintained. We have shown that as soon as this object is accomplished, unless the infection has lasted too long and has become too great, there occurs a fall of temperature and recovery from the disease.

The amount of serum necessary, therefore, will vary in each case. At present we have no means of deciding in the individual case what the primary dose should be. Neufeld, on the basis of the theory of *Schwellenwert*, decided that in the average man the dose should be about 75 cc. We also think the dose should be large and, from our experience and from the observations concerning neutralizing substances in the blood of infected patients, we have established more or less arbitrarily an initial dose of our standard serum of 90 to 100 cc. The frequency and size of the succeeding doses must be regulated largely by the effects obtained from those preceding. It was hoped that it might be possible to regulate the succeeding doses by determining the antibody content of the patient's blood at frequent intervals. This procedure is too complicated to be carried out regularly, even in hospitals that are best equipped for such work. In others this method is absolutely impossible. We must therefore be guided by the experience already gained from the careful study of a series of cases. We have concluded from these studies that it is extremely important that once having started specific treatment, it should be continued until a definite favorable result has been obtained and, in order to accomplish this, the serum should be given every 8 hours in doses of 90 to 100 cc. unless there are indications to the contrary. Our experience has indicated that the total amount of serum required may be diminished by active and persistent treatment at the start. The average total amount of serum required in our cases treated during the past winter has been about 250 cc.

In a considerable number of cases, within 20 minutes to an hour following the injection of serum there occurs an elevation of temperature, followed by a marked fall. This is described in the following pages as the so called thermal reaction. If the temperature continues low and the patient's condition is good, no further administration of serum is necessary. These patients, however, must be very carefully watched. The temperature should be taken every 2 hours and, if the temperature during the next 24 hours rises to 102°F. or over, a second dose of serum should at once be administered. If no fall of temperature occurs following the first dose, or if it does not fall to 102°F. within 8 hours, the second dose of serum should then be given. It is obvious that this rule should not be absolutely hard and fast, for the temperature is not the only guide to the patient's condition. However, it is better to err on the side of giving the serum too soon than of delaying too long.

If, following the second dose of serum, no reaction occurs, within 8 hours another similar dose should be administered, and this should be repeated as often as necessary. The patient should be observed day and night and the treatments should be given at night as well as by day whenever necessary. In the cases in which no reaction is obtained after several doses of serum, great care must be exercised to detect the presence of complications such as empyema or otitis media, to which the persistence of temperature may be due and on which the failure of the serum to act depends.

Serum Reactions.

The injection of serum may be followed by several types of reaction; first, the true anaphylactic reaction, second, the so called thermal reaction, and finally, usually at a later period, the symptom complex called serum sickness or serum disease.

Anaphylactic Reaction.—In individuals who are sensitive to horse serum, there may develop at once or within 15 or 20 minutes after the introduction of 40 to 100 cc. of serum, a more or less severe asthmatic attack with dyspnea and flushing of the face, followed by cyanosis, sweating, cough, and general anxiety, and an urticarial eruption. Methods have previously been described by which these

reactions can be avoided. According to Walker (31), however, reactions may occur, though very rarely, even in patients who have not reacted positivelyto horse serum and who have received a subcutaneous desensitizing dose. He has found that these individuals, though not sensitive to horse serum by the usual test, are very sensitive to the proteins in horse hair and horse dandruff. In these unusual cases small doses of horse serum produce no reaction, but doses of over 40 to 50 cc. of serum seem to induce a true anaphylactic attack. In these cases the first dose of serum usually produces complete desensitization and, following a second dose, even a very large one, no reaction of this type occurs. An anaphylactic reaction, unless extremely severe, is usually quickly relieved by the hypodermic injection of 0.6 cc. of epinephrin (1: 1,000) solution or 0.5 mg. of atropine sulfate, or both.

It is the consensus of opinion among those who have had large experience in the administration of serum that severe anaphylactic reactions occur with great rarity, even when precautions have not been taken to avoid them. If all the precautions we have mentioned are taken, serious anaphylactic shock will probably never occur. The administration of antipneumococcic serum should never be neglected or omitted because of the remote possibility of the occurrence of these reactions.

Thermal Reaction.—Another type of reaction, that which follows the intravenous injection of various foreign proteins, the so called thermal reaction, appears usually from 20 minutes to 1 hour after the injection, and is characterized by chilly sensations or a general shaking chill, slight difficulty in breathing, and cyanosis. The temperature rises rapidly 1–3°F. and then falls, often to normal. During this fall there may be profuse perspiration. The patient usually rests quietly after a reaction and the general physical appearance seems improved. These reactions apparently have nothing to do with anaphylaxis or previous sensitization and may occur following the first dose of serum or following subsequent doses. Frequently the temperature remains normal, but in other cases after 6 to 24 hours the temperature again becomes elevated. It is this type of reaction which sometimes occurs following the administration of large doses of vaccines, and in such cases has been thought by some to have therapeutic value. We have

never felt that this reaction in itself is of benefit to the patient and believe that it should be avoided if possible. In certain instances administration of the serum too rapidly or the administration of cold serum may be responsible. Little treatment for this type of reaction need be given, reassurance, the application of heat to the extremities, and warm drinks being sufficient.

Serum Disease.—Following the administration of foreign serum, there frequently occurs a group of symptoms which together are called serum disease or serum sickness. The symptoms are fever, skin rashes, most frequently urticaria or erythema, edema of the skin, general glandular enlargement, and pains in the joints, and the symptoms may appear individually or in combination. The typical or most characteristic serum disease consists of all or most of the symptoms appearing together 7 to 14 days following the administration of serum, the attack lasting a few days to a week or more. The attacks may recur one or more times at intervals of a few days to a week or even longer. Such a typical and fairly well marked disease is not the rule, however. In most cases the course is extremely irregular. Certain of the symptoms, chiefly the skin rashes, may appear very early, within a day or a few days following the administration of serum. Later other symptoms may appear, singly or together. And these symptoms may reappear or persist irregularly for a month or more. Not all cases receiving serum show the symptoms. Mild symptoms of the disease appear in only about half of the treated cases, severe attacks only rarely or in not more than 10 per cent of the cases. The severity of the symptoms is not directly proportional to the amount of serum received, though undoubtedly the severe cases are more likely to occur in the patients that have received large doses of serum.

The fever in the mild cases may be very slight, hardly noticeable. In the severe cases it may be high, even reaching, exceptionally, 104° or 105°F. The cases with fever may cause much anxiety to the physician, since he is frequently in doubt as to whether the serum reaction is responsible for the fever or whether the fever is due to some complication. During the entire period of convalescence the physician should always be on his guard and, while he should realize that serum sickness may be accompanied by marked symptoms and

elevation of temperature, he should remember that it is unsafe to ascribe all unusual symptoms occurring during this period to serum disease. When fever occurs, frequent Roentgenographic examinations should be made to detect the presence of delayed resolution or the appearance of fluid in the chest. The occurrence of otitis media or localized infection should be looked for carefully. If the fever is due to serum disease, the patient does not usually appear very ill, as would be the case if the fever were associated with some focal complication with pus formation. The pulse is relatively less rapid, sweating is uncommon, and the appetite is good. The leukocyte count often helps in the differential diagnosis. While the total leukocyte count may be moderately elevated in serum sickness, up to 15,000 to 20,000, there is usually an increase in the relative number of lymphocytes, 30 to 40 per cent, and frequently the number of eosinophils is slightly increased, 3 to 7 per cent.

The skin rash may consist of only a few urticarial wheals appearing intermittently, or it may be widespread and consist of a diffuse erythema, even scarletiniform, or it may be more irregular, suggesting measles. When the urticaria is extensive, pruritus may be very marked. This is usually the most distressing symptom. It may be relieved to some extent by allowing the patient to have a bottle of calamine lotion by the bedside which he applies to the itching areas as desired. Some such formula as the following may be used:

Carbolic acid	2 cc.
Calamine	4 gm.
Zinc oxide	8 "
Glycerol	12 cc.
Lime-water	16 "
Water up to	120 "

With this lotion the patient can usually be kept fairly comfortable during the day. At night the use of epinephrin hypodermatically, in doses of 0.6 to 1 cc., has been found useful. Following the injection the urticaria disappears like magic for a few minutes to a few hours, when it again returns. If one uses epinephrin at night, the patient will often fall asleep as soon as the urticaria disappears and will sleep several hours before being disturbed by the itching.

Occasionally there may be considerable edema present with the skin rash, or it may occur independently. It is most common about the face and neck and it may be so extensive as to close the eyes. This usually lasts only a few hours.

During the course of serum sickness there usually occurs slight enlargement of the superficial lymph glands. Sometimes only a few of the glands are enlarged; at other times the enlargement is general. The spleen may also become palpable, though this is not the rule. Occasionally the cervical glands beneath the sternomastoid muscle may become so enlarged and tender as to suggest a pyogenic infection, but this condition usually completely disappears in 2 or 3 days.

There is rarely nausea or vomiting. Very rarely acute abdominal pain may be complained of. There is no localized tenderness or rigidity, however, and this condition is usually transitory.

During the serum sickness, as von Pirquet and Schick (22) have noted, a disturbance of kidney function may be present. Longcope (11) found that about 10 per cent of his carefully studied cases showed albuminuria and hyaline and granular casts in the urine. There is little change in the coefficient of urea excretion or in the phthalein output. The excretion of water and chlorides is, however, profoundly affected, both being rapidly diminished with the onset of the sickness. This change in renal function is transitory and the return to normal is usually rapid.

Accompanying the other symptoms there may develop stiffness in the joints. The process seems to be extra-articular. There is rarely, if ever, any fluid in the joints, nor is the skin hot or reddened. There may be a slight amount of edema about the joints. All the joints may be attacked or only one or two. A joint frequently affected is the temporomandibular, and this causes considerable annoyance to the patient. Usually the joint symptoms pass away in a few days, but may last a week. The administration of aspirin or sodium salicylate and sodium bicarbonate usually gives relief from the discomfort. 0.3 to 0.6 gm. of the salicylate with 1 gm. of sodium bicarbonate may be given every 4 to 6 hours. Occasionally the patient complains of a headache which may persist for several days, but this is usually relieved by aspirin.

As previously stated, serum sickness may be very mild or it may be prolonged and troublesome, and the joint pains and urticaria may cause considerable distress. As far as we know, however, it is never serious, and leaves no permanent bad effects. Von Pirquet and Schick, whose monograph contains the best description of the disease, report no fatal cases, and we have never seen any serious effects, even in the worst cases. The fact that serum sickness may possibly occur should never deter the physician from giving serum. If life can be saved, the occasional occurrence of troublesome symptoms in a few patients is of little importance.

Supplementary Therapeutic Measures.

General Measures.—In addition to the specific therapy advised in the treatment of Pneumococcus Type I infections, the general hygienic and therapeutic management of the case must not be neglected. It is extremely important that the patient should be nursed, rather than nurse himself, from the onset of the infection. The prognosis in individuals who have pursued an ambulatory course during the first few days of the disease is distinctly worse. If transfer to a hospital is to be made, the patient should not be allowed to dress and walk to the ambulance, no matter how mild the infection may seem. Where impossible to move in the horizontal position, the transfer may be made semi-reclining in a chair. Even this exertion is often exhausting. The patient should not be allowed to help himself to food or drink, or to bedpans. The strict enforcement of these precautions plays an important part in the patient's recovery.

Diet.—The diet during the acute course of the disease is relatively unimportant. In general it should be fluid, easy to swallow and digest. Purées and soft boiled eggs, in addition to liquids, may be given if the patient desires them. In some cases whisky in small amounts may be beneficial. Water, 3,000 cc. or more a day, should be conscientiously given.

Treatment for Abdominal Distention.—The routine use of a daily morning enema of soap-suds aids in preventing this complication. Distention most frequently occurs in the severely intoxicated cases. It can frequently be controlled by proper diet, stupes, and medi-

cated enemata. If abdominal distention occurs, milk should be temporarily eliminated from the diet. Stupes may be conveniently given by applying compresses soaked in olive oil mixed with turpentine, 1 part of turpentine to 3 parts of oil, and covering with flannels wrung out in as hot water as the patient can tolerate, the heat being retained by a thick pad. The hot flannels should be changed frequently for a period of 20 minutes. A rectal tube may be inserted to aid in the passage of gas. This entire procedure is repeated at 20 minute intervals. To be effective, the application of stupes must be continued for a long period. Following their application, medicated enemata may be used. A very effective enema is one composed of ox gall 4 cc., turpentine 8 cc., asafetida 12 cc. This combination is diluted in 1 to 2 pints of soap-suds and is followed in 1 hour by a soap-suds enema. Usually this is effective in relieving the distention, at least for a short time. Some cases are temporarily benefited by the hypodermic use of pituitrin in doses of 0.5 cc.

Digitalis.—The experimental work of Porter and his coworkers (23) tends to show that the heart muscle is not vitally injured in pneumonia and that respiration usually fails before the circulation. Cohn (R17) has found that the pneumonic heart can be influenced by digitalis in quite the same way and by approximately the same dosage as the normal heart. Consequently it has been our custom to use some form of digitalis as a routine. On account of the convenience of administration and its accurate standardization, digipuratum has been used at the Hospital of The Rockefeller Institute, though any standardized form of digitalis may be used in corresponding doses. It is extremely important that the use of digitalis should be commenced early in the disease in all patients, in order that they may be partly digitalized before an immediate need arises. The intravenous use of digitalis or strophanthin is thereby avoided. The intravenous injection of these drugs may give rise to very serious symptoms, especially if the patient has previously received digitalis. Under this condition these drugs should never be injected into the vein. If patients are treated early, they are given 1 gm. of digipuratum at the rate of 0.5 gm. a day by mouth. If they are not seen until late in the disease and appear quite ill, 1 gm. is given on the first day. With this dosage there is usually evidence of the heart muscle being digitalized in about 24

hours. If the patient's condition does not indicate its further use, the drug is discontinued. If, however, occasion arises for digitalis later in the disease, it is again given at the rate of 0.5 gm. a day until the indication is satisfied, but it is not usually continued beyond 2 gm. The beneficial effects of the use of digitalis are best seen in cases of collapse, in which the pulse rate often rises to 150 to 180. This rise is usually due to flutter or fibrillation of the auricles. In these cases digitalis acts by blocking the irregular and frequent auricular impulses, so that often the ventricular rate falls below the initial rate and the symptoms of collapse disappear.

Optochin.—In 1911 Morgenroth and Levy (12) reported the discovery of optochin (ethylhydrocuprein), a derivative of quinine, which was found to have a specific bactericidal action on pneumococci both *in vitro* and *in vivo*. The experimental studies led to great hopes that this drug might be found to have specific curative effects in patients suffering from pneumonia. Extensive studies have been made on the drug in The Rockefeller Institute, both experimentally on animals by Moore (R43, R44, R45) and in the treatment of patients by Moore and Chesney (R12, R13). To our disappointment, however, in the treatment of 75 cases we have observed no definite beneficial effects. Because of this experience and owing to the danger of the drug producing injury to the eyes, the routine use of this drug in the treatment of acute lobar pneumonia cannot be recommended.

Results of Serum Treatment.

We shall here discuss only the effects of the administration of specific serum in the cases of lobar pneumonia due to infection with Type I pneumococcus.

General Effects.—We have already discussed the so called thermal reaction which may immediately follow the administration of serum. The fall of temperature associated with this reaction may be accompanied by amelioration of symptoms and the whole picture may present the features of a natural crisis. This occurs, however, in a small number of patients only. In other patients, following the fall of temperature and the relief of symptoms which almost always

accompanies it, there may occur a return of symptoms and rise of temperature, though, if serum is again promptly administered, this practically never reaches its previous height. In other cases there is little immediate effect of the serum on the temperature or pulse. In almost all cases, however, very soon after the administration of serum the patient's general condition improves. This is not only subjective, but is evident from the patient's better mental condition and increased interest in his surroundings. The cyanosis becomes less, and the pulse rate frequently falls. This phenomenon strongly suggests that, in addition to its other effects, antipneumococcic serum has a detoxifying effect. The exact nature of this effect however, can only be surmised. Beginning with this general improvement, unless the infection has been overwhelming, there occurs a gradual lowering of temperature and pulse rate, and amelioration of the other symptoms, the patient usually returning to a normal condition within a few days. What almost certainly happens following the administration of the serum is that the progress of the disease is stopped. How rapidly complete recovery may occur depends on numerous factors. That the progress of the disease is stopped if sufficient serum is given is made evident by the following facts, in addition to those just mentioned: First, in practically all cases, following the administration of the serum, there has been no extension of the local lesion. In certain cases in which the serum has been administered early, sometimes only a very small portion of a lobe has become consolidated. Second, invasion of the blood by pneumococci has been prevented (page 35). Slight invasion of the blood by bacteria probably always occurs at some time during the disease, at least in all but the very mild cases. In the more severe cases a true septicemia is usually present, and this always occurs in the cases ending fatally. In our opinion the fatal outcome is usually dependent upon this general infection. Now, in almost every case in which we have administered serum and in which the blood culture was positive before the administration of the serum, one dose of serum, or at the most two, has resulted in the blood becoming sterile. This fact seems to us of great importance in judging the efficacy of the serum. Third, following the administration of the serum, there appear in the patient's blood the immune

bodies which normally appear only at or about the time of crisis. It is possible, of course, that these immune bodies are only those contained in the serum administered. Whether the patient himself is stimulated or permitted to produce immune bodies more rapidly or in greater numbers than would otherwise be the case is not known.

We have previously stated (page 33) that the presence of precipitable substance in the urine is a bad prognostic sign. In several cases where the reaction was positive at the time the administration of serum was commenced, following the administration of serum the reaction became negative.

Effect on Resolution and the Development of Complications.—The administration of serum apparently causes no change in the rate of resolution of the lung tissue already involved. This process is apparently independent of immunity reactions, and consolidation of the lung may persist even after the temperature is normal and the patient is, from a superficial examination, well. There is no evidence, however, that the occurrence of resolution is delayed or interfered with by the administration of serum. Complications, such as empyema, may occur even after the employment of serum. In certain cases the complications have already been present when the administration of serum has been commenced; in others the complication has undoubtedly appeared after this time. From a theoretic standpoint and from what we have learned from animal experiments, we might expect that the frequency of focal infections would be increased when serum treatment is employed. Infections in susceptible animals are most likely to be focal when infection has occurred after the animals have been partially immunized, either actively or passively. Therefore if patients with severe infections which would otherwise be fatal are insufficiently treated with serum, it might be expected that, though the general infection is overcome and life is saved, focal infections might occur. Our statistics, however, do not show any increased frequency of complications among the treated cases. The non-specific treatment of cases with complications is exactly the same as though no serum had been given. Our empyema cases have all been operated upon and all except one have recovered.

Effect on Mortality.—The final test of the effectiveness of immune serum must rest on the evidence concerning the saving of lives. Although the number of cases so far treated in the Hospital of The Rockefeller Institute is not large, the results obtained have been so striking that, taken in connection with the other evidence presented, and supported, as they are, by the observations of others, they indicate almost certainly that the serum as employed by us has a marked curative effect in the treatment of pneumonia of Type I. Up to the present time 107 cases of lobar pneumonia of this type have been treated with serum, and of these but 8 have died, 7.5 per cent. When this is contrasted with the mortality of 25 to 30 per cent, as observed in our cases before we commenced serum treatment and in the cases observed elsewhere, the results obtained are definite and striking. During the past 5 years we have treated with serum every Type I case coming under our observation unless the case has been very mild or unless the patient was admitted at a time when recovery had obviously commenced. That the more severe cases were treated with serum is shown by the fact that among 105 treated cases, the blood cultures were positive before treatment was commenced in 40, or 38 per cent, while of 34 cases admitted during the same period and in which no serum treatment was given, only 2 showed positive blood cultures, or less than 6 per cent.

In the fatal cases we have included every patient that received serum, even a single dose. A brief statement as to these fatal cases may be of interest. Three of the cases were treated only a few hours before death, when they were desperately ill. One of the patients recovered from the pneumonia, but died on the 16th day following a pulmonary embolism. One patient was treated late in the disease and later suffered from numerous complications, empyema, abscesses, etc., and finally died on the 54th day from a general streptococcus infection. One patient had an extensive tuberculous involvement of both lungs, with a very small area of fibrinous pneumonia in a part of one lobe. The blood culture became negative and he apparently recovered from the pneumococcus infection, and death was probably due to the tuberculous disease. This leaves but two cases in which the serum could possibly have been of use or in which any form of specific treatment could probably have saved the lives. One of

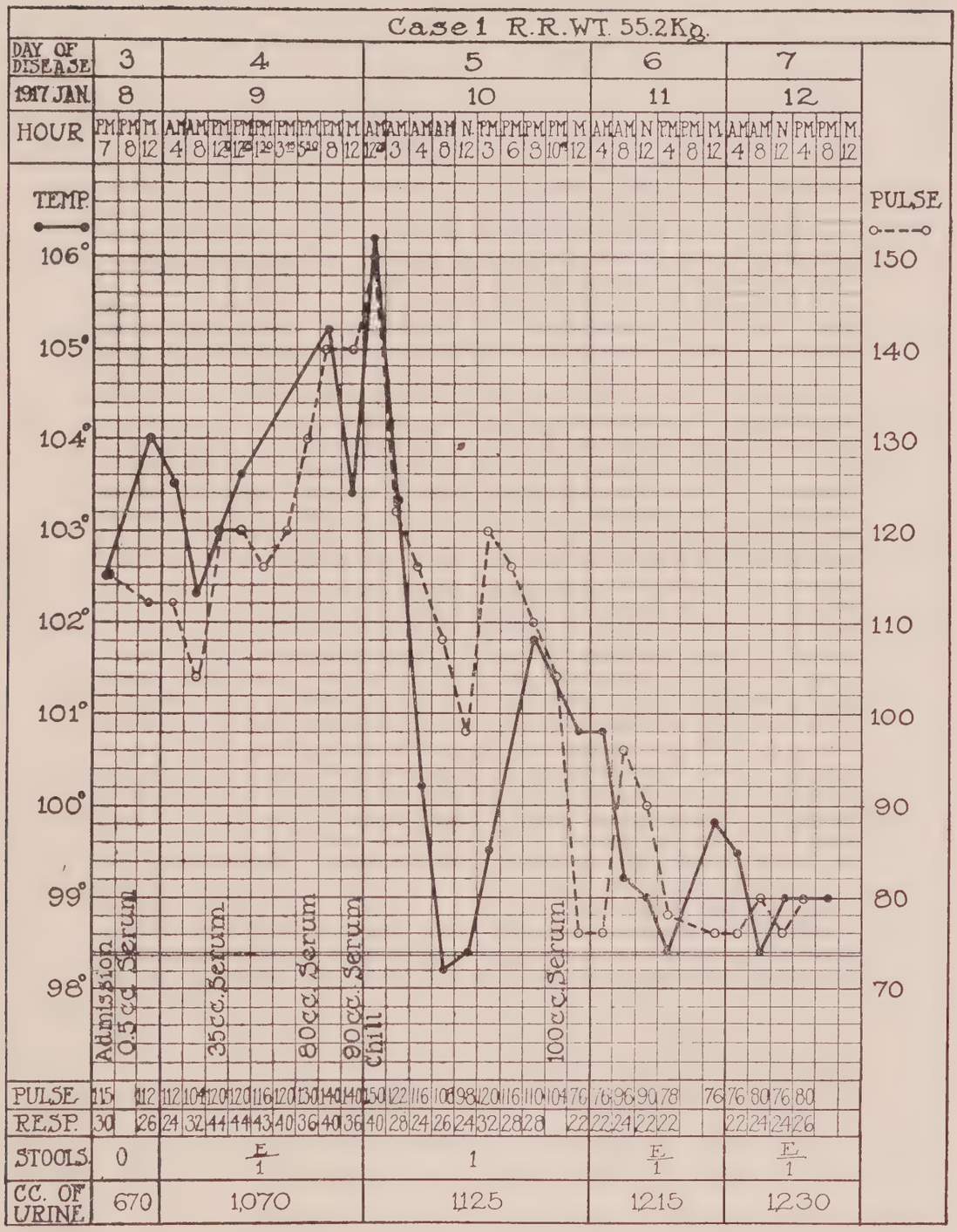
these cases was among the first ones treated by us and from our present knowledge was probably treated insufficiently. He was treated on the 5th and 6th days, dying on the latter day. The other case was one showing an extremely high grade of blood infection, 300 colonies per cc., when he was admitted on the 6th day. In spite of this, with very vigorous serum treatment, he lived until the 12th day, but finally succumbed.

The results obtained by others in small series of cases in hospitals in New York, Boston, and Pittsburgh, where the treatment could be carried out with proper attention to details, have also been definite and satisfactory. Last fall on the Mexican Border Major Nichols (18) treated 63 cases occurring among the soldiers. Of these 63 cases, 5 died, a mortality of 8 per cent. Among 18 cases with Type I infection not treated with serum, 7 died, a mortality of 39 per cent. From his experience Major Nichols concluded that "no patient with Type I infection who dies without the early intravenous administration of large doses of Type I serum can be said to have received the best treatment."

Cases Illustrating the Method of Treatment with Immune Serum.

The following abstracts of cases and temperature reactions illustrate some of the statements that have been made regarding treatment and serum reactions.

Case 1.—R. R., age 22 years, student (Text-fig. 3). This patient was admitted January 8 at 7 p.m., suffering from pneumonia involving the left lower lobe. The onset had been quite typical, with chill 48 hours before admission. He was moderately sick, temperature 102.5°F., pulse 115, respirations 30, leukocytes 31,000. Shortly after admission 0.5 cc. of horse serum was injected subcutaneously, but no previous intradermal test for serum sensitiveness had been made. The sputum was bloody; a small amount was at once injected into a mouse. The following morning tests made of the growth in the peritoneal cavity of the mouse showed that the patient was suffering from an infection with Type I pneumococcus. The blood culture taken on admission was positive, the plate showing 1 colony per cc. At 12.17 the intravenous injection of antipneumococcic serum was commenced. Although the serum was given slowly, after he had received about 35 cc. of serum he had some signs of serum intoxication, suffusion of face, some respiratory difficulty, and he vomited several times. The administration of serum was therefore discontinued. He complained of tightness in the chest



TEXT-FIG. 3. Temperature chart, Case 1.

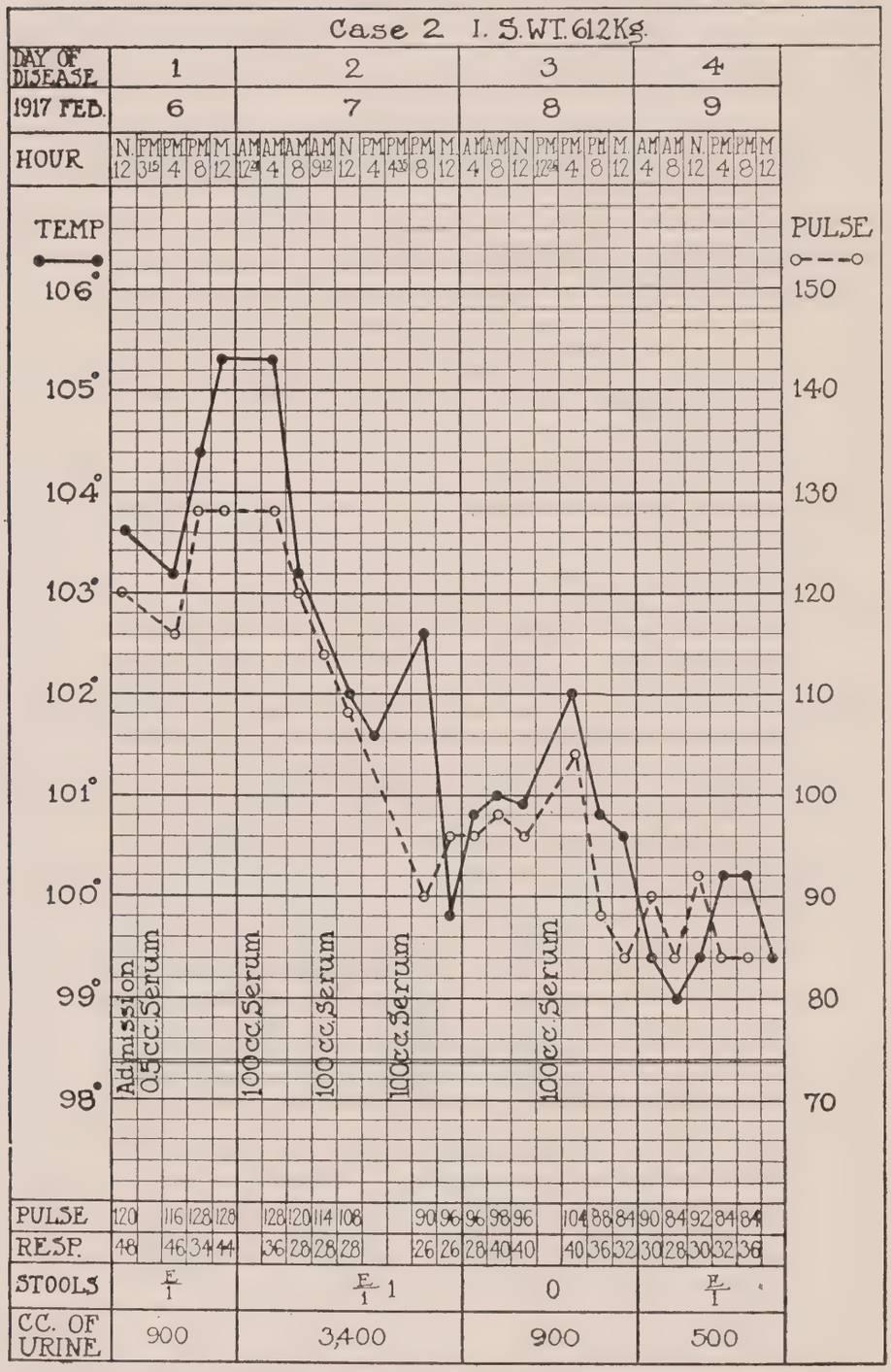
and a feeling of general weakness. 1 cc. of a 1: 1,000 solution of epinephrin was injected intravenously and the patient's condition soon returned to normal, except that he sweated profusely. He was somewhat alarmed at what had taken place. His condition did not materially change during the afternoon, and at 5 p.m. serum was again administered, this time 80 cc., without any untoward symptoms. During the evening no definite change occurred in his condition. His temperature, however, had risen to 105.2°F. at 8 p.m., pulse 140, respirations 40. Therefore at 12 midnight another injection of 90 cc. of serum was made. Following this injection the temperature rose to 106.2°, pulse 150, respirations 40. He had a shaking chill during which he became cyanosed. The temperature fell rapidly during the night and at 8 a.m. it was only 98.2°, pulse 108, respirations 26. His color was good but he seemed rather weak and exhausted. During the day his condition was excellent, but the temperature gradually rose, reaching 101.8° at 8 p.m. He was then given another dose of serum, 100 cc. There was no reaction of any kind following this injection. The temperature the following morning was 99.4° and he felt quite well. There was no extension of the process in the lungs and following this the patient made a good recovery. During convalescence, however, he had quite severe serum sickness, the symptoms beginning 6 days following the last injection and some persisting for 2 weeks. There occurred persistent urticaria, stiffness and pain in the joints, edema of the face, and albumin and casts in the urine. He had some fever from January 20 to January 30, the temperature reaching 103° on January 23. However, he made a good recovery and was discharged on February 7 perfectly well, without a trace of albumin in the urine.

This patient illustrates well certain of the untoward effects of the administration of serum. It is probable that if an intradermal skin test had been made on admission, the presence of sensitiveness to serum would have been detected. As it was, he was incompletely desensitized, and he had a mild anaphylactic reaction. This, however, was not serious, and that he was completely desensitized following it is shown by the absence of reaction after the subsequent dose. Following the second large dose of serum there occurred what we have termed a thermal reaction. This was unusually severe, but very characteristic. It is possible that the following dose of serum might have been omitted or at least somewhat delayed, but it is better to have erred by giving it too soon than by delaying too long. The serum sickness was severe, one of the most severe cases we have seen. Repeated and frequent tests of the agglutinating power of the patient's serum were made in this case and, following the first dose of the horse serum, the patient's serum acquired agglutinating

power which increased following the second dose, and this increase persisted. If we had observed only this case, conclusions as to the benefit of the serum would be difficult. Judging from the fact that the blood culture was positive before the serum was given and that the patient was well, as far as the pneumonia was concerned, on the 6th day, it seems probable that the disease was shortened. In any case he recovered, and while the symptoms due to the serum were somewhat distressing, they were justified, when the results in the whole series of cases are considered.

Case 2.—I. S., age 32 years (Text-fig. 4). This patient was admitted about 8 hours following the initial chill. There were definite signs of involvement of both lower lobes and he presented all the characteristic features of acute lobar pneumonia. His temperature on admission was 103.6°F., pulse 120, respirations 48, leukocytes 35,000. He appeared seriously sick; the blood culture taken on admission was positive. Immediately after admission a specimen of sputum was injected into a mouse, and the diagnosis of the type of infecting organism, Type I, was determined within 12 hours. He was then given his first treatment. The temperature at this time was 105.2°, pulse 128, respirations 44. There was no immediate reaction following this treatment, but the next morning the temperature was lower, 103.2°, and his general condition was improved. Owing to the obviously serious nature of the infection, the treatment was continued very actively, 100 cc. being given at 9 a.m. and 100 cc. at 4.30 p.m. No marked reaction followed either of these injections, though after the injection in the evening the temperature rose slightly, but fell during the night and was 101°F. at 8 a.m. At this time he was very bright; the signs of intoxication had all disappeared. No increase of lung involvement had occurred. In spite of this he was watched carefully during the day and, as his temperature was rising, he was given another dose of serum. Following this his temperature fell. His blood was tested frequently for agglutinating power. This, which was negative before the first dose of serum, reached a high level immediately following, and this level increased with subsequent doses. He also had severe serum sickness, beginning very early, 3 days following the last dose of serum, and lasting for a week, with all the symptoms, including joint pains. On the 4th day of the serum sickness the temperature reached 103.8°. He made a good recovery and was discharged on the 24th day of the disease.

This case well illustrates the importance of early diagnosis and treatment. In this case we feel that the treatment was carried out in a perfectly satisfactory manner and, except for the serum sickness, the results were all that could be hoped for. The administration of the fourth dose on the 3rd day, we think, was of great importance in



TEXT-FIG. 4. Temperature chart, Case 2.

preventing any possible recurrence of the infection and spread of the lesion.

The above cases, while favorable ones, illustrate the method of treatment and the results in a large proportion of the cases. In certain cases one or two doses of serum have sufficed; in others more have been necessary. In a number of cases no effect on the temperature curve followed the first few doses. It is therefore important that, having once commenced the serum, its administration should be actively pushed until favorable results are obtained. It is now evident that no harm can result from this procedure and, judging from our experience, much good.

PREVENTION OF PNEUMONIA.

Epidemiology.

Lobar pneumonia is generally considered to be an endemic and sporadic disease. It is habitually present among the population of certain parts of the world, showing, however, certain seasonal variations in morbidity. In spite of the fact that its incidence may reach at times a very high rate, the relation, in origin, of one case to another has never been very apparent. Diseases of typical epidemic character, on the other hand, usually spring from a limited source, attain widespread proportions often through traceable channels, and, then, after a limited time, subside, perhaps not to recur again for a period of years. Such differences between endemic and epidemic diseases, however, probably depend more upon the facility of infection and the susceptibility of the soil on which the disease is implanted than upon any essential difference in the mechanism of communication. From a public health standpoint, therefore, it is just as important to attempt to understand and control the channels of communication of an endemic as of an epidemic disease.

Lobar pneumonia was recognized as a distinct clinical entity in the time of Hippocrates. In the 16th and 17th centuries widespread epidemics are reported to have occurred, in which the character of the disease seems to have been somewhat different from that observed in modern times. In the 19th century pneumonia assumed a definitely endemic character. At present it is a common infection throughout the United States and Canada. It is frequent all over temperate Europe, and in the inhabited portions of the south temperate zone such as Australia, parts of South America, and South Africa. Although it is much less frequent in the tropics, it is often seen there among the inhabitants of the plateau regions. Small epidemics in limited communities such as military camps, schools, prisons, and on shipboard are not of infrequent occurrence, probably due to the intimate contact among individuals which such a life entails. In recent

years two epidemics of more widespread proportions have developed. One occurred during the course of construction of the Panama Canal, in which the disease affected principally the colored laborers brought to the Isthmus from the West Indies. The other took place in the Rand Mining District in South Africa. Here, as in Panama, the incidence of the disease was by far the greatest among the black boys who were imported from Central Africa to labor in the mines. These two epidemics were probably dependent upon the same factors, the great susceptibility to pneumonia of individuals coming from regions where acute respiratory infections were uncommon and the intimate contact dependent upon living in common quarters such as barracks and compounds.

The census of 1900 showed that in the United States somewhat over 10 per cent of all deaths were due to some variety of pneumonia; this, of course, includes both bronchial and lobar pneumonia, the latter being responsible for by far the greater number. Some statistics seem to indicate that the incidence of pneumonia is increasing. It is probable that this apparent increase may be due to better methods of diagnosis and recording. One may safely say, however, that the incidence of pneumonia has shown no tendency to diminish. This may be due in part to the general acceptance of the non-contagious character of lobar pneumonia and the consequent lack of adequate measures of prevention. During the same period of time such diseases as diphtheria and tuberculosis have shown a definite decrease in morbidity, and one feels inclined to ascribe this shrinkage to the widespread activity directed toward the limitation of these diseases. It is certain that until the causative factors that operate in the continued widespread incidence of lobar pneumonia have been brought to light, and until such facts as have been discovered receive practical application by Boards of Health, the continued yearly frequency and high mortality of lobar pneumonia will not be checked.

Pasteur once said that it is possible to eradicate all diseases of bacterial origin, but that first one must know the bacterial agent and record the means by which it establishes itself in the human body. In the case of pneumonia, the greatest difficulty that stands in the way of obtaining this knowledge is the paradoxical fact that a large proportion of normal individuals may constantly harbor in their

mouth secretions organisms which are apparently identical with those causing the disease. This situation, however, is not characteristic of pneumonia alone, for somewhat similar conditions are found in other diseases. For instance, the colon bacillus, an organism not far removed from the typhoid bacillus, normally inhabits the intestines of all healthy human beings, and certain cocci resembling the meningococcus, the cause of cerebrospinal meningitis, are found in the mouth secretions of healthy individuals. In these instances it has been an easy matter to separate the disease-producing bacteria from the more or less harmless saprophytes of normal mucous membranes by simple bacteriological methods such as cultural and fermentative reactions. The problem in pneumonia, however, has been a much more difficult one, since by cultural methods it is impossible to differentiate between the more harmless pneumococci and the definitely pathogenic ones. However, by the employment of the more specific immunity reactions, indications of differences between these two general groups of pneumococci have been obtained.

In view of the fact that a majority of healthy individuals harbor in the mouth an organism which has been, until the present time, indistinguishable from the pneumococcus causing pneumonia, the presumption has been fairly general that most pneumonic infections are to be considered autoinfections and that the important factor in determining the incidence of the disease is variation in susceptibility brought about by exposure or other accidental occurrences. If this assumption is true, no useful results can be hoped for from the usual preventive measures employed in other diseases which are designed to limit the spread of the infectious agent. At the Hospital of The Rockefeller Institute (R3, R51, R52) during the past few years an attempt has been made to find out whether in reality the development of the majority of cases of lobar pneumonia depends upon infection with the pneumococcus which the individual habitually carries in his mouth secretions, the immediate onset being caused by some factor which temporarily lowers resistance, or whether contact infection, either direct or indirect, plays a part of some importance. In order to throw light upon this question, the problem has been approached from several different points of attack. In view of our ability to recognize, by means of specific reactions, definite types of

disease-producing pneumococci, it has been deemed important, in addition to confirming the constancy of these types, to study also the character of the pneumococci present in the normal human mouth. In addition to the study of the differences between the pneumococci occurring during disease and those in the normal mouth, the character of the pathogenic types has been followed during the disease and convalescence, the time of disappearance from the mouth secretions of the pneumococci causing the disease noted, and the type of pneumococcus by which they were supplanted studied. The mouth secretions of a large number of healthy individuals in contact with cases of lobar pneumonia of known type have also been studied, in order to determine whether in the mouths of such individuals the disease-producing types of pneumococcus occur with greater frequency than in normal mouths and, if so, whether this may be attributed to the intimate association, the purpose being to detect the existence of actual healthy carriers of pathogenic types of pneumococcus. The type of pneumococcus present in the mouth secretions of a large number of healthy individuals in whom no history of contact with lobar pneumonia could be obtained, has also been carefully studied. The pneumococcus of the mouth has been isolated by injecting a white mouse intraperitoneally with about 0.5 cc. of saliva, which is a sufficient quantity to kill the animal if pneumococcus is present. The organism has subsequently been isolated in pure culture from the heart's blood of the mouse and the type determined by means of the agglutination test.

Types of Pneumococcus in the Mouths of Normal Persons.—In Table IX the results of a study of 297 normal individuals are given, showing the percentage of healthy people who habitually carry pneumococcus in their mouths, and also a classification of the type of pneumococcus found. For purposes of comparison the percentage incidence of the different types of pneumococcus in 454 cases of lobar pneumonia is also given (Table X).

In Table IX is presented the study of the mouth secretions of 297 individuals in whom no history of contact with an acute or recent case of lobar pneumonia could be obtained. From the mouth secretions of 116 of these individuals pneumococci were isolated, whereas from the mouth secretions of 181 no pneumococcus was obtained. Of the 116 individuals carrying pneumococcus in the mouth at

TABLE IX.

Distribution of Different Types of Pneumococcus in the Mouths of Normal Persons.

Type of pneumococcus.	Incidence.	
		per cent
I	1	0.8
II	0	0.0
IIa	1	0.8
IIb	7	5.8
IIx	14	11.6
III	34	28.1
IV	64	52.9
Pneumococcus present	116	
" absent	181	
	297	

TABLE X.

Incidence of Types of Pneumococcus in Lobar Pneumonia.

Type of pneumococcus.	Incidence.	
		per cent
I	151	33.3
II	133	29.3
IIa	6	1.3
IIb	4	0.9
IIx	9	2.0
III	59	13.0
IV	92	20.3

the time of observation, the different biologic types of pneumococcus were found in the following percentage of instances: Type I was found once, an incidence of 0.8 per cent; Type II was found not at all; the Subtypes IIa, IIb, and IIx were found 22 times, a percentage incidence of 18.2. Of these Subtype II organisms, Type IIa is the least common and Type IIx the most frequent. This distribution of the variants of Type II parallels the distribution of the larger, more inclusive pneumococcus types in the normal mouth. Type III, or *Pneumococcus mucosus*, was found 34 times, a percentage incidence of 28.1. The immunologic studies of Type III have shown that this organism is one that shows little or no variation in specificity and in this it corresponds to the typical pneumococci of Type I and Type II. On the other hand, it presents a paradox

in that it is frequently found over long periods of time in the mouths of healthy individuals and, in spite of this frequency, it is the cause of the smallest number of acute cases of lobar pneumonia of the four major types of pneumococci. Type IV was encountered in normal saliva 64 times, a percentage incidence of 52.9. This type of pneumococcus is therefore the dominant type in the mouth secretions of normal individuals.

Consideration of Table X shows that in a series of 454 cases of lobar pneumonia, Pneumococcus Types I and II was present in 284 instances, a percentage incidence of 62.6. The dominance of these two types of organisms in disease shows a striking contrast to their almost complete absence in the mouth secretions of normal individuals. The Subtypes IIa, IIb, and IIx were responsible for 19 instances of disease, 4.2 per cent. Type III pneumococcus, despite its frequent occurrence in the normal mouth, was responsible for but 59 instances of disease, 13.0 per cent. Type IV pneumococcus, the commonest pneumococcus present in the bacterial flora of the normal mouth, was responsible for 92 of the cases of lobar pneumonia studied, or 20.3 per cent.

Comparison of these two tables shows that the pneumococci most commonly found in the mouth secretions of normal individuals give rise to a minority of the cases of lobar pneumonia. The disease produced by these organisms, with the exception of Type III, is less severe in character, indicating a lower grade of pathogenicity of these types for man. On the other hand, Types I and II cause a majority of cases of lobar pneumonia, are of high virulence for human beings, and are seldom or never found in the mouth secretions of normal individuals who have not been in intimate association with cases of lobar pneumonia. This seems to indicate that lobar pneumonia due to Types I and II does not arise from infection with a pneumococcus which is habitually carried in the mouth, but that infection with these organisms occurs from without.

Types of Pneumococcus in Sputum of Pneumonia Patients.—If all cases of lobar pneumonia were due to infection of the individual with a type of pneumococcus which was more or less constantly present in his mouth secretions, one would expect this organism to persist or at least reappear in the sputum after recovery from the disease had occurred. Therefore, a study has been made of types of pneumococcus present in the mouth during the disease, as well as those present during convalescence. After the type of organism responsible for the disease has disappeared, the type of pneumococcus present later has been determined.

In Table XI are presented the results of a study in 15 cases. These 15 cases are representative and form but a small part of the total number of cases studied.

TABLE XI.

Persistence during Convalescence of the Type of Pneumococcus Causing the Disease.

Case No.	Type of pneumococcus during height of disease.	Day of disease on which observation was made, reckoned from day of initial chill.	Type of pneumococcus during convalescence.
2,456	I	14	No pneumococcus.
		27	" "
2,467	I	31	" "
2,527	I	10	I
		24	No pneumococcus.
2,550	I	25	IIa
		32	IIa
2,571	I	9	No pneumococcus.
		16	IV
		23	IV
2,477	II	6	II
		13	IIb
		21	IIb
		29	IIb and IV
2,536	II	10	II
		17	No pneumococcus.
		25	" "
2,543	II	11	" "
		18	" "
		31	IV
2,586	II	8	No pneumococcus.
		22	" "
2,611	II	2	II
		9	IV
		16	IV
		23	IV
		30	No pneumococcus.
		37	IV
2,465	III	11	III
		13	III
		20	IIx
		27	IV
		34	IIx and IV
		40	IIx " IV

TABLE XI—*Concluded.*

Case No.	Type of pneumococcus during height of disease.	Day of disease on which observation was made, reckoned from day of initial chill.	Type of pneumococcus during convalescence.
2,488	III	4	III
		14	IV
		21	IV
		28	IV
2,550	III	14	III
		21	IV
2,689	III	7	III
		14	III
		21	III
		28	III
		35	III
2,640	III	7	No pneumococcus.
		14	" "
		21	" "
		28	" "

A study of Table XI reveals the fact that only in exceptional instances does one find in the sputum for a considerable length of time after recovery the same type of pneumococcus with which the individual was infected during the disease. The exact time at which the disease organisms disappear varies. They have disappeared as early as 7 days from the date of onset of the disease, but have been present as late as 90 days after recovery. Usually they have disappeared in 3 to 4 weeks. In a number of instances in which the disease organism has persisted for an unduly long period of time, delayed resolution or some chronic respiratory condition has been present. After the disease organism disappears, either no pneumococcus is present or the disease type has been supplanted by one of the types which are usually found in the saliva of normal individuals. These observations indicate that the type of pneumococcus responsible for lobar pneumonia in any individual is but a temporary and accidental inhabitant of his respiratory passages, and, therefore, give additional evidence that infection occurs from without. They also indicate the length of time that convalescents may be a source of infection to others in case the mode of infection is by contact.

It is seen from Table XI that Type III pneumococcus, which is known to occur frequently in the normal mouth, disappears in the majority of instances from the mouth flora of cases of pneumonia as rapidly as do pneumococci of Types I and II, which induce disease more frequently. This suggests that Type III pneumonia may also be communicable but that, owing to the frequent occurrence of this organism in the normal mouth, the lines of communication may be impossible to trace.

Pneumococcus Carriers.—Infectious diseases, aside from those spread by insects, usually spread by immediate contact, either with a person suffering from the disease, or with a convalescent, or with a healthy carrier. The importance of these modes of transmission varies with the different diseases and in many instances more than one of these mechanisms may be involved. Infection in lobar pneumonia by immediate contact with a person ill of the disease undoubtedly occurs, and we have seen a number of instances in which this mode of infection was apparently responsible. With most diseases, however, the healthy carrier may be a greater menace to the community than the infected individual, largely because of failure to recognize the carrier condition. In the epidemiology of certain diseases, notably typhoid and epidemic cerebrospinal meningitis, the importance of the carrier state is well recognized. In Table XII evidence is presented to show that a considerable percentage of persons intimately associated with patients suffering from lobar pneumonia harbor in their mouths pneumococci of the same type as those causing the disease. The persons who have been studied have been relatives or friends, especially those who have taken care of the patients. We have already shown that pneumococci of Types I and

TABLE XII.

Incidence of Carrier Condition in Healthy Individuals in Contact with Cases of Lobar Pneumonia.

Type of pneumococcus in patient.	Total no. of contacts examined.	No. of positive contacts.	
			per cent
I	160	21	13.1
II	149	18	12.1

II are not found in the mouths of normal individuals not associated with cases of pneumonia.

For purposes of comparison with Table XII, the incidence of Types I and II in the mouth flora of normal individuals unassociated with cases of lobar pneumonia is given (Table XIII).

TABLE XIII.

Incidence of Pneumococcus Types I and II in Saliva of 297 Healthy Individuals Not in Contact with Cases of Lobar Pneumonia.

Type of pneumococcus.	Incidence.	
		per cent
I	1	0.33
II	0	0.00

A study of Table XII shows that of 160 healthy individuals in contact with cases of lobar pneumonia of Type I, 21, or 13 per cent, carried in their mouth secretions at the time of examination a pneumococcus of the same type. Of 149 individuals in contact with cases of Type II pneumonia, 18, or 12 per cent, were found to carry Pneumococcus Type II. When these figures are compared with those from 297 healthy individuals not in contact with lobar pneumonia, in whom the incidence of Type I pneumococcus was 0.3 per cent and that of Type II was 0.00 per cent, very little doubt can be entertained that through contact with cases of lobar pneumonia due to Type I or Type II pneumococcus, healthy carriers arise with considerable frequency. In almost every instance in which a carrier of a Type I or Type II pneumococcus has been observed, the type of organism in the carrier has corresponded with that of the case with which he has been associated. Carriers of Type I and Type II pneumococcus harbor these organisms in their mouth secretions for variable periods of time, the average being from 3 to 4 weeks. At the end of this time the disease-producing type usually disappears and is replaced, if pneumococci are present at all, by one of the types of pneumococcus found in the saliva of individuals who have not been in contact with cases of pneumonia.

Types of Pneumococcus in Dust.—The possible occurrence of pneumococcus in dust has been known for some time. Little signifi-

cance, however, has been attached to this fact. In order to determine whether pneumococcus could be recovered with any regularity from dust, and what types are present, Stillman (R52) has carried out the following study. Specimens of dust were collected as follows: A piece of paper was wrapped about a small scrubbing brush, this in turn was covered with a piece of cloth, and the whole sterilized in the autoclave. The dust of the room to be studied was swept up with the sterile brush from an area about a foot square. The specimen, folded in the sterile paper, was taken to the laboratory where it was mixed with 1 to 1.5 cc. of broth, and the mixture injected into the peritoneal cavity of a white mouse. If the animal died cultures were made from the heart's blood and the type of organism responsible for its death was determined. In all, 62 specimens of dust from rooms in which no case of pneumonia was present were examined. Pneumococci were recovered from 18 of these specimens, or 29 per cent, and were absent in 44, or 71 per cent. The frequency of occurrence of the different types of pneumococcus is shown in Table XIV.

TABLE XIV.

Types of Pneumococcus Recovered from Dust of Rooms in Which Lobar Pneumonia Had Not Occurred.

Type of pneumococcus.	Incidence.	
		per cent
I	1	5.5
II	0	0
IIa	0	0
IIb	4	22
IIx	3	16.6
III	2	11
IV	8	44.4

From Table XIV it is seen that the types of pneumococcus usually recovered from the rooms in which healthy individuals are living correspond, as one would expect, to those found in the mouth secretions of these individuals. On the other hand, the strictly disease-producing types of pneumococcus, Types I and II, are not found unless possibly a healthy carrier is present, as was the case in the one instance given in the table.

A similar study was made of the dust of rooms in which cases of Type I or Type II pneumonia had occurred. In all, 183 specimens of dust were examined. In 109 of these pneumococcus was not found. In 74 of the specimens of dust pneumococcus was present; Table XV gives the distribution of the various types recovered.

TABLE XV.

Types of Pneumococcus Recovered from Dust of Rooms in Which Cases of Lobar Pneumonia Due to Type I or Type II Pneumococcus Had Occurred.

Type of pneumococcus.	Incidence.	
		per cent
I	25	33.8
II	23	31.1
IIa	0	0
IIb	2	2.7
IIx	2	2.7
III	2	2.7
IV	20	27.0

This study shows that pneumococcus is more frequently present in the dust of rooms in which lobar pneumonia has occurred than in those in which no pneumonia has existed, and under the former circumstances pneumococci of Types I and II are frequently found. Moreover, in these instances the type of pneumococcus found in the dust corresponded with the type of pneumococcus causing the disease.

Conclusions Regarding Epidemiology.—When all these observations are taken together, we have considerable evidence, contrary to the opinion previously held, that pneumonia, in a considerable proportion of the cases at least, arises by infection from without. This evidence relates mainly to the cases due to infection with Type I or Type II pneumococcus, which organisms, however, are responsible for over 60 per cent of the cases. Pneumococci of these types persist for a limited time only in the mouths of patients who have suffered from the disease, and are very rarely, if ever, present in the mouths of normal persons who have not been in immediate contact with such patients. Pneumococci are not infrequently found in dust, but those of Type I and Type II are practically never found except in the environment of persons sick of the disease or in the environment of

carriers. Possible sources of infection of those who acquire infection with pneumococci of these types, therefore, are other patients suffering from pneumonia due to the same type of pneumococci, persons who carry these organisms during convalescence, persons who have acquired the organisms by close contact with patients (healthy carriers), and dust from the immediate environment of patients or carriers.

We therefore now have evidence in regard to the mode of infection in pneumonia of these two types which affords a justifiable basis for instituting preventive measures. The conditions in regard to the other types of pneumonia are not yet sufficiently well known to justify discussion here.

General Preventive Measures.

If lobar pneumonia in a large percentage of instances is readily communicable from one individual to another, as the evidence given above seems to indicate, it is clear that public health authorities should take cognizance of this fact in their efforts to limit the widespread incidence and high mortality of this disease. Even if accurate knowledge of the exact mode of transmission were unknown, the enforcement of such precautions as are taken in the effort to control the spread of tuberculosis, epidemic cerebrospinal meningitis, and diphtheria would be justifiable. Since we have definite knowledge concerning the mode of transmission in a large percentage of the severe cases of lobar pneumonia, it is extremely important that these precautionary steps should be taken.

One of the essential measures for the success of a campaign against any communicable disease is to provide the public with all the available knowledge concerning it and to explain the dangers that each infected individual presents to those with whom he comes in contact. The first step, therefore, in the control of lobar pneumonia would seem to be the institution of an educational campaign, so that the public mind may be receptive to such measures of control as may be deemed necessary.

In order that public health boards may institute the measures necessary for the control of an epidemic disease, it has been found essential that they should obtain knowledge of the incidence of the

disease in a locality, and of the focal distribution of the cases. The only means by which this knowledge can be acquired is through compulsory notification. Certain Boards of Health now have regulations requiring notification of cases of lobar pneumonia, but these are rarely enforced, and at present the incidence of this disease can only be approximately determined by calculation from the mortality statistics. Such a measure would not, in all probability, meet with public opposition, as has developed from the attempt to bring about compulsory notification of tuberculosis or venereal disease. Because of the ease and rapidity with which the diagnosis of lobar pneumonia is made by the practitioner, the statistics obtained would probably be quite accurate. In view of the fact that only certain types of lobar pneumonia have been shown to be communicable, public health laboratories should be equipped to determine the type of pneumococcus responsible for each particular case of the disease. Additional data concerning the epidemiology of the disease would then be available.

Each case of pneumonia should be regarded as a focus for the spread of the infection and the care of each patient should include those measures which have been found serviceable in other communicable diseases. The patient should be isolated as far as possible and his communication with other individuals should be reduced to a practical minimum. Inasmuch as the pneumococcus finds its way to the outside world mainly in the secretions from the buccal and respiratory tracts, the sputum should be collected in special containers and either burned or disinfected. All utensils, handkerchiefs, bed clothing, etc., which are likely to become contaminated through contact with the mouth or otherwise should be sterilized before again being put into general use.

The studies of Stillman have shown that a large percentage of pneumonia patients contaminate the rooms which they occupy, as is shown by the large number of instances in which strictly pathogenic types of pneumococcus can be recovered from the dust of these apartments. This indicates the necessity for thorough cleansing of any room occupied by a case of pneumonia at the close of the period of convalescence. In addition, the daily cleansing of the sick room should be practised in such a way as to avoid dissemination of dust particles.

From the studies presented above it is seen that two types of carriers of pathogenic pneumococci exist: first, convalescents from the disease, and second, healthy individuals who have not contracted the disease themselves, but who have been in intimate association with cases of the disease. These individuals usually carry the pathogenic pneumococci for 3 to 4 weeks, but instances have been observed in which the pneumococci have been harbored in the mouth secretions for much longer periods. The control of healthy carriers of disease-producing bacteria is one of the most difficult and perplexing problems in the regulation of infectious diseases, and one which has not as yet been satisfactorily solved. The ideal method would be to isolate and limit the contact of carriers until they no longer harbor the organisms of disease. Such a procedure unfortunately is not always practicable. In any case carriers of the strictly disease-producing types of pneumococci should be instructed to avoid promiscuous spitting, they should be told to refrain from kissing, and they should be instructed in the proper methods of disposing of any sputum or expectoration. Disinfecting mouth washes have proved of some advantage in eliminating the organisms from the nasopharyngeal secretions of meningococcus carriers, and it is possible that a similar method may prove of service in shortening the period of time in which convalescents from lobar pneumonia or pneumococcus carriers harbor the pathogenic organisms in the mouth. This procedure has not yet been carefully tried. In convalescents it is not likely to prove of great value, however, because the pneumococci are probably present in the deeper air passages not reached by the disinfectant.

It is well known that epidemics of lobar pneumonia often arise and attain considerable proportions in small communities in which people live in close association with one another, such as military barracks, labor compounds, schools, asylums, hospitals, prisons, etc. It is under these conditions that the preventive measures described above should prove of the greatest value. In the control of an epidemic disease it is necessary that prophylactic measures should be instituted coincidently with the development of the first cases, in order to prevent the wide dissemination of the virus and the consequent failure of all precautionary methods. Therefore, upon the

outbreak of pneumonia in such communities as military barracks, all patients should be immediately isolated, their contact with other individuals cut off, and a search for carriers of the disease-producing types of pneumococcus made among their associates. The carriers found should be isolated, or at least made as harmless as possible, by instituting the measures mentioned above. It is only in this way that the distribution of the pneumococcus and the resulting high prevalence of pneumonia can be prevented.

Prophylactic Vaccination.

Animal experiments have shown that it is very easy to produce active immunity to pneumococci by the injection of small doses of dead organisms, even in animals as susceptible as the mouse and rabbit, and this immunity persists for a considerable time. It is theoretically possible, therefore, to immunize men to the fixed types of pneumococci by the injection of dead cultures. The advisability of doing this in a civilian population under ordinary conditions has never been seriously considered. There are undoubtedly marked differences in the susceptibility of individuals to pneumococcus infection, just as there are differences among individuals in susceptibility to diphtheria. The so called Schick test has made the detection of individuals who are immune to diphtheria possible. If a similar method were applicable in pneumonia, so that persons either susceptible or immune to pneumococcus infection could be readily determined, the number whom it would be necessary to immunize could be greatly reduced, and probably the immunization of the entire population against infection, with certain types of pneumococci at least, would be both practical and possible.

Even in the absence of methods of differentiating the susceptible individuals from the immune, however, whenever pneumonia exists to an extraordinary degree among certain groups of individuals, and in the case of epidemics, the question of preventive inoculation should be considered. The only place, to our knowledge, where this has been attempted on a large scale has been among the workers in the mines in South Africa, where the disease prevails to a most alarming degree and causes the death of very large numbers of native workmen. These efforts were made during 1911 and 1912 under the direction of

Sir Almroth Wright (35). In spite of the many conclusions and hypotheses presented in the elaborate report of this work, no definite results as to the efficacy of the procedure were obtained. No effort was made to immunize the men against the different specific types of pneumococci; indeed no attention was paid to differences in type, and it is therefore evident that the work could only result in confusion. Later Lister (9, 10) carried on experimental studies in the South African Institute for Medical Research, both on animals and man, and established certain facts which may go far in making this method of prevention of pneumonia one of great value. He first determined the types of pneumococci causing the prevailing pneumonia, and learned that, in addition to the types found in Europe and the United States, at least one additional type exists, which is one of those most frequently encountered there. He then studied the production of active immunity to these various types in animals, and also investigated for the first time the best method of producing immunity in man. He has found that it is easier to produce immunity by intravenous than by subcutaneous injection. However, by the injection of sufficiently large doses of vaccine he has been able to produce immunity in man by subcutaneous injection. The tests of immunity employed consisted in demonstrating the presence of agglutinins and opsonins in the blood. It may be objected that these methods do not necessarily indicate active immunity. However, as far as animal studies may be applicable, it has been the experience of all investigators that animals whose blood contains these antibodies are always actively immune. We therefore believe that these tests are perfectly justifiable and reliable in judging of the presence of active immunity. As we have stated elsewhere, estimations of protective power probably give more accurate quantitative indications of immunity, but in the present circumstance such great accuracy is not important. From Lister's studies he concluded that for prophylactic purposes "three subcutaneous inoculations, at 7 days' interval, should be employed; each dose should consist of 6,000 million cocci of each group against which immunity is desired." He found that 8 months after the last inoculation his own serum still contained agglutinins and opsonins against the types of organisms injected. He has found that the reactions following the injection of even

very large doses of vaccine are slight. Our experience fully bears this out. These observations of Lister are of great value and importance. Our studies on the production of immunity in animals (R31), however, suggest strongly that more effective and rapid immunity may be produced by the frequent injection of small amounts of vaccine than by the infrequent injection of large amounts. Such a method of immunization, however, involves practical considerations, the discussion of which cannot be entered into here. The important fact is that active immunity may be produced against the specific types of pneumococci and that this immunity persists for a considerable length of time. The question of the advisability of active immunization against pneumonia is now a very acute and important one, since within a short time large numbers of susceptible men will be collected together in soldiers' camps, and it is probable that, unless preventive measures are instituted, large numbers of them will be attacked by the disease. Before preventive inoculation is undertaken on a large scale, certain questions must be answered: Is it justifiable or practicable to subject large numbers of men to the resulting inconvenience and loss of time in the hope of protecting a number of them from this serious disease? If this method is employed, how may the highest grade of immunity be produced, what should be the size of the dose, the site of injection, the frequency and number of injections, and what is the best form of antigen to be employed? A number of these problems are now being studied. The results of such a method of prophylaxis cannot, of course, be foretold with accuracy. We can only say that as far as experiments on animals are of value, the employment of this method would result in great saving of human life. A trial of this method in army camps has already been strongly recommended by Major Nichols (18) of the United States Army.

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EXPLANATION OF PLATES.

Plate 1.

Fig. 1. Requirements for sputum inoculation of mouse.

Fig. 2. Method of grasping mouse for inoculation.

Plate 2.

Fig. 3. Intraperitoneal inoculation of mouse.

Fig. 4. Method of collecting peritoneal washings from sputum-inoculated mouse.

Plate 3.

Fig. 5. Injection of serum.

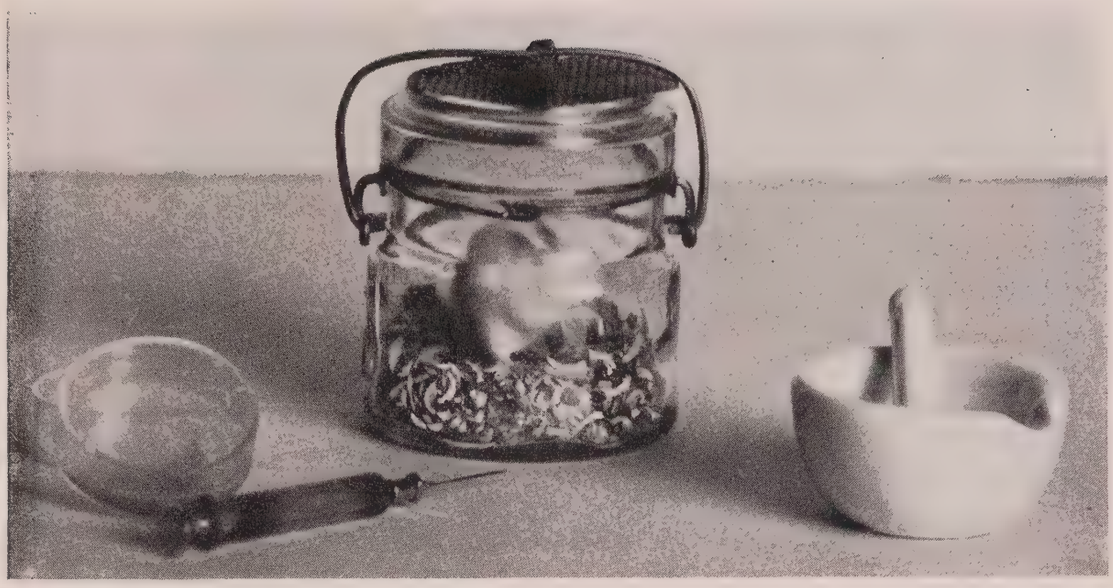

FIG. 1.

FIG. 2.

(Avery, Chickering, Cole, and Dochez: Acute lobar pneumonia.)

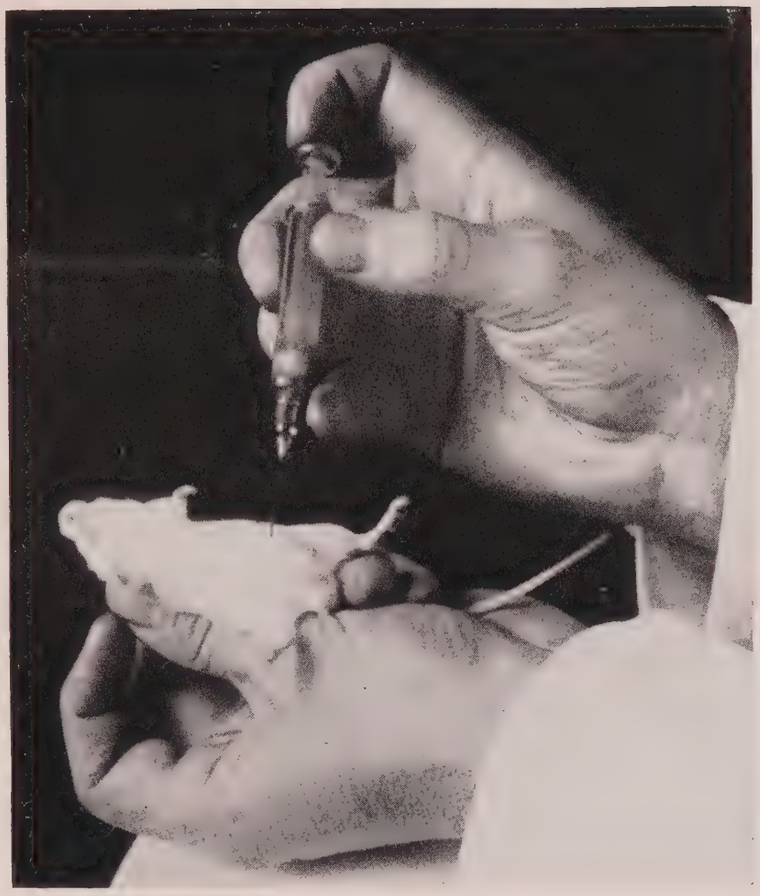

FIG. 3.

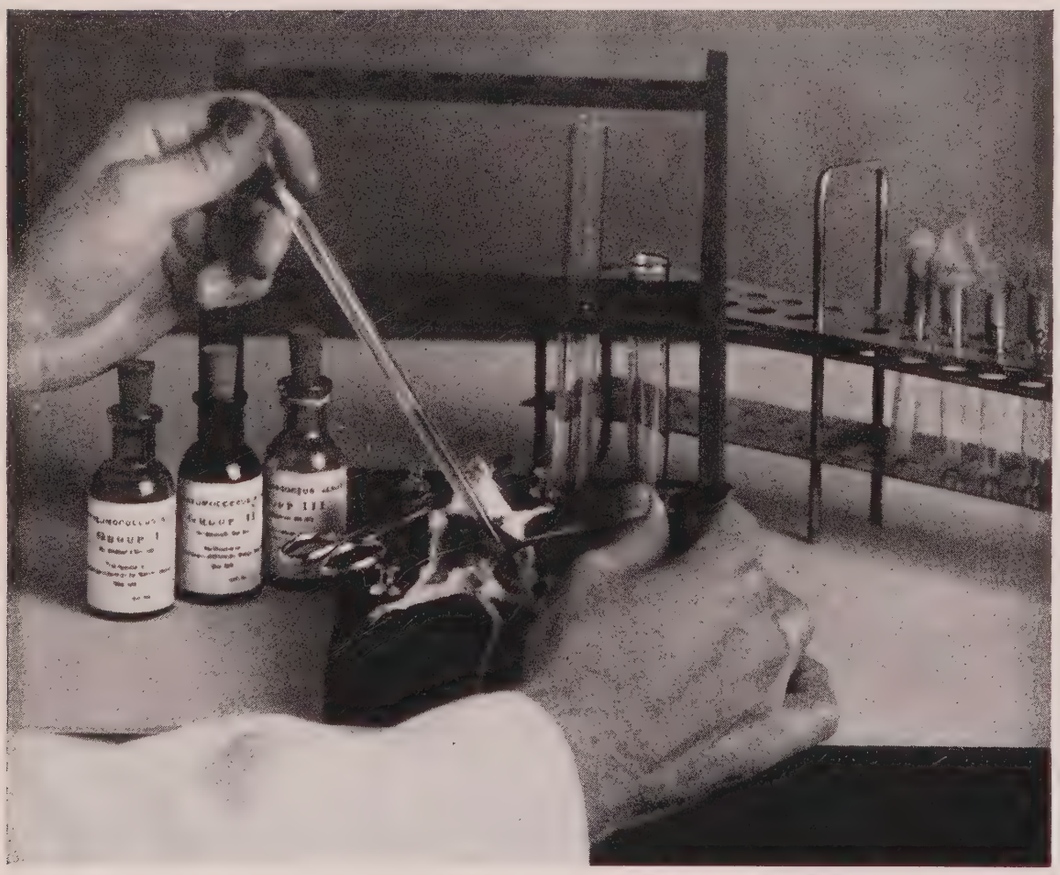

FIG. 4.

(Avery, Chickering, Cole, and Dochez: Acute lobar pneumonia.)

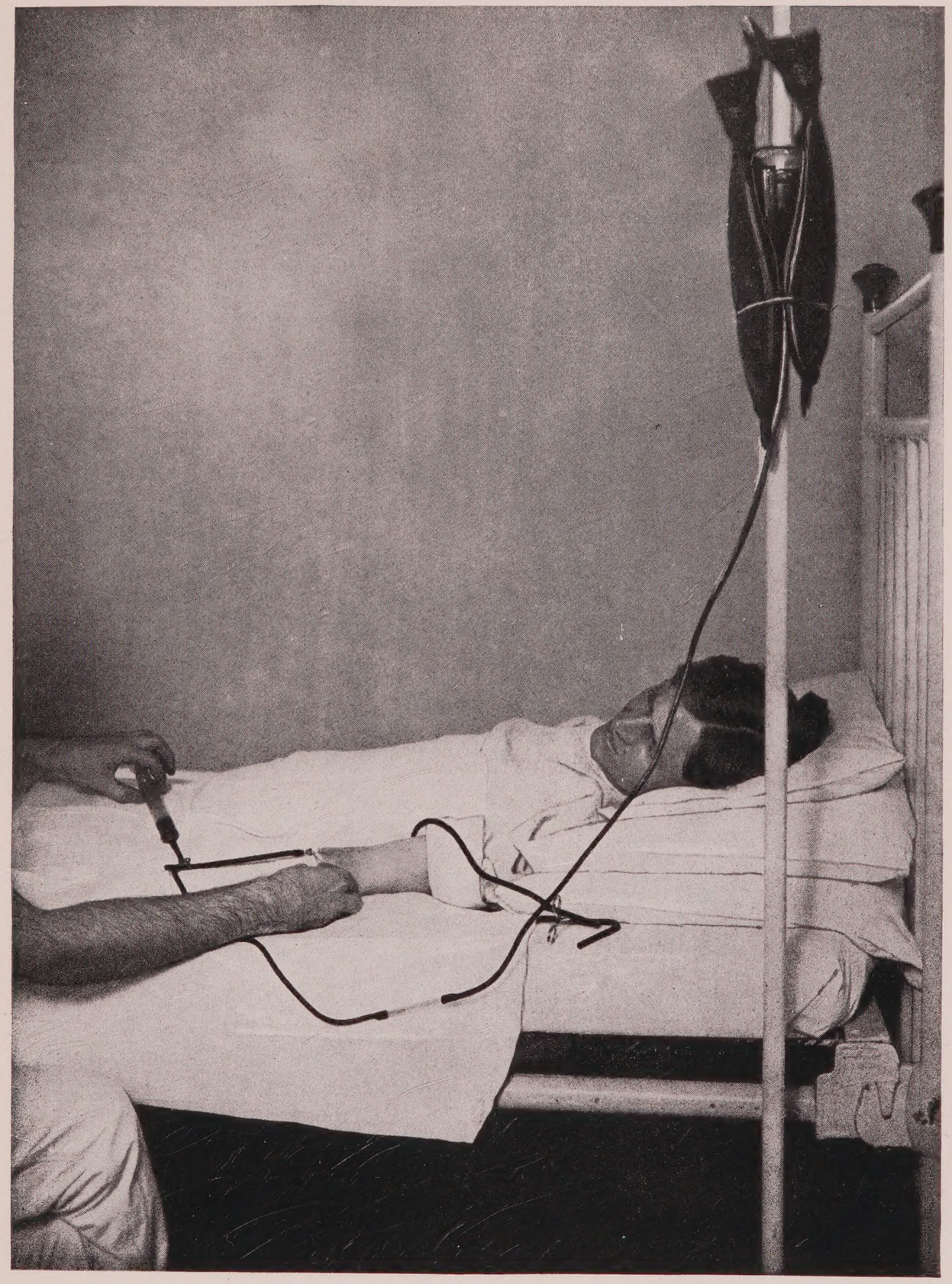

FIG. 5.

(Avery, Chickering, Cole, and Dochez: Acute lobar pneumonia.)

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